### P-001

**Characteristics of Hemolytic Uremic Syndrome (HUS) Associated with Enterohemorrhagic**

**Escherichia coli** **Infection, Japan, 2008**

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**Introduction and Objectives:** Enterohemorrhagic *E. coli* (EHEC) is a highly pathogenic subgroup of Shiga toxin–producing *E. coli* (STEC) that can lead to hemolytic uremic syndrome (HUS) and a potentially life-threatening disease. This study describes the epidemiological characteristics of HUS due to EHEC in Japan.

**Material and Methods:** EHEC infection has been a notifiable disease since August 1996 in Japan. The case definition of a confirmed case requires both isolation/detection of the organism and confirmation of Verocytotoxins (VT) in addition to compatible clinical manifestations (e.g., bloody diarrhea, fever or HUS). Since April 2006, the HUS case definition has been changed as follows; a clinical HUS with either VT detection or positive serum antibodies to STEC serogroups.

EHEC infection data are stored in the National Epidemiological Surveillance of Infectious Diseases (NESID) system. The NESID system records, in addition to the actively tracked information collected from local government through a questionnaire by e-mail, were reviewed for HUS cases in 2008; the incidence by age group, VT type of O157 and routes of infection.

**Results:** A total of 2818 EHEC infections meeting the case definition were identified in 2008. The *E. coli* serogroups included O157 (70.3%), O26 (16.5%), O111 (4.2%), O103 (2.0%) and others (7.0%). Of these, 94 had HUS. The attack rates (ARs) by age group were 6.6% for children aged 0–4 years, 4.5% for those 5–9 years, 2.8% for those 10–19 years and 0.4% for those 20–29 years old. Children aged 0–4 years had a significantly increased risk of incidence (odd’s ratio: 3.3, 95% CI: 2.2–5.0). The O-antigen serotype of O157 and routes of infection.

**Conclusions:** HUS seemed to be predominantly associated with VT2-producing *E. coli*. HUS cases were concentrated in children under 10 years of age. Therefore, control measures for HUS to this age group are very important. These should include not feeding younger children liver sashimi or undercooked meat and preventing secondary transmission at home and in nurseries. As the infection source for a half of HUS cases is unknown, more rapid investigation of cases is necessary. The high CFR and the severe consequence for HUS were observed. We should raise awareness of the severity of HUS.

### P-002

**Outbreak of Shiga Toxin-Producing**

**Escherichia coli** **O111 Following Consumption of a Raw Beef Dish, Yukhoe, Occurred in Japan in April 2011**

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**Background:** In April 2011, a Shiga toxin-producing *Escherichia coli* (STEC) O111 outbreak occurred in Japan, in which 181 people became ill and five died. The outbreak included 32 (17.7%) cases of hemolytic uremic syndrome (HUS), which were mostly associated with acute encephalopathy. The source of the food poisoning outbreak, which occurred in Toyama Prefecture and also in three other prefectures, was traced to a chain of barbecue restaurants. The outbreak resulted in the deaths of five people, in each of whom there was a shorter incubation period than usual. The infections resulted from consumption of a raw beef dish called as ‘yukhoe’, which is served at this chain of restaurants.

**Results:** Microbiologic analysis: Diagnoses of STEC were performed by microbiological laboratories in a hospital or public health center. Almost all patients were infected mainly with STEC O111 (stx2/eae+), but the isolated STEC exhibited a diverse combination of serotypes and toxin types. There were two serotypes, namely, O111 and O157. serotype O111 harbored one toxin type (stx2), whereas serotype O157 harbored three toxin types (stx1, stx2, and stx1, 2). In addition, *E. coli* O111 isolates lacking the stx2 gene, O111 (stx-), were detected from some patients. Furthermore, no isolate with the stx2 gene was detected even by a PCR survey of more than approximately 800 O111 colonies cultured from one patient. No STEC were detected by fecal examination in some patients with HUS. Serological investigation: The serum samples taken during the acute phase of HUS in cases without any STEC isolates or *E. coli* O111 (stx-) showed IgM antibodies only to the LPS of O111. Therefore, this outbreak of food poisoning was considered to have been caused by STEC O111. PFGE analysis: Pulsed-field gel electrophoresis (PFGE) for STEC O111 isolates demonstrated that the band pattern of the stx2 and stx(-) isolates of *E. coli* O111 differed by only one band, which was considered to be the same outbreak strains. The STEC O117 isolates with various patterns were detected, but all strains were considered to be related outbreak strains.

**Conclusions:** In this outbreak, a diversity of STEC isolates was detected, however, the cause of the outbreak was considered to be STEC O111 (stx2+). Further investigations are necessary to be clarified.
P-004

Enterohemorrhagic *E. coli* O111 Outbreak Associated with Raw Beef Consumption in a Barbecue Restaurant Chain, Japan

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Introduction and Objectives: An outbreak of the Enterohemorrhagic *E. coli* O111 occurred in Japan in April–May 2011. On 26 April 2011, Takaoka Public Health Center (PHC) in Toyama prefecture received a case suspected of EHEC and the person had visited the Takaoka branch of a barbecue restaurant chain A (BBQ chain A). In the next day, the center confirmed EHEC O111 in patient’s stool culture. Since then, multiple PHCs and local governments in Toyama and neighbouring prefectures received reports of not only EHEC O111 and/or O157 but also several cases of HUS from customers of several BBQ chain A restaurants. The EHECs’ strain had the same pattern for PFGE, and that confirmed occurrence of widespread outbreak. The Toyama prefectural government requested the Field Epidemiology Team of NIID to start the investigation on 4 May. Our main objective is to perform a thorough investigation to reveal the full picture of this outbreak.

Material and Methods: Records of clinical information and food consumption regarding BBQ Chain A were collected through the PHCs’ investigation, based on the Infectious Disease Control Law and the Food Sanitation Act. Laboratory tests were operated by the Toyama Prefectural Institute of Public Health. We conducted a case-control study. The case was defined as a person presenting with one symptom (nausea, abdominal pain, vomiting) or diagnosed with HUS AND isolated EHEC O111 or/O157. The control was a person who had eaten at the BBQ chain A but didn’t have any symptoms (nausea, abdominal pain, vomiting) or diagnosed with HUS AND isolated EHEC O111 or/O157 or a detected O111 antibody. The control was a person who had eaten at the BBQ chain A but didn’t have any symptoms and didn’t detected any EHECs. Risk factors for the EHEC infection were estimated by logistic regression analysis.

Results: We had 89 confirmed cases and 146 controls. The ratio of females in cases were 53% (47/89). The onset date were distributed between 19 April and 7 May, the date with highest numbers of patients were 26 and 25April (19 cases). The date that patients visited restaurants were distributed between 17 and 24 April, and 40 cases ate at the specific branch on 23 April. Most cases had symptoms such as diarrhea (89%) or abdominal pain (80%). The median age of cases was 20 years old (range: 1–70). There were 32 HUS cases (36%); and five of them were fatal (case fatality rate: 6%). Among cases that had detailed consumption records, a raw beef menu was consumed by 100% (82/82) and that was significantly associated with illness (OR = 59.8, 95% CI: 8.1–441.5).

Conclusions: Our investigations identified the raw beef menu as the most likely vehicle of this outbreak, but lack of records for trace-backs prevented us to point out the precise source of infection. Consumers were notified the risk of raw meat by this incident and that lead the national government to revise and tighten regulations for meat providers and restaurants that serve raw beef.

P-005

VTEC non-O157 Serotypes Linked with Haemolytic Uremic Syndrome in Finland (1996–2011)

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Introduction and Objectives: Hemolytic uremic syndrome (HUS) is one of the most severe clinical manifestations of verocytotoxin-producing *Escherichia coli* (VTEC). The incidence rate of human VTEC infections has been low in Finland (0.3–0.9/100 000/year 1996–2009). Majority of the isolated strains belong to the serotype O157:H7/H- and the remaining strains to 32 other O-groups. In this study, we report all the detected non-O157 serotypes which were linked to haemolytic uremic syndrome (HUS) in Finland.

Material and Methods: In Finland, clinical microbiology laboratories throughout the country are required to submit all human VTEC isolates to the Bacteriology Unit (THL) for confirmation and for further phenotypic and genotypic characterization. The data of symptoms were asked from isolate sending laboratory by phone. The presence of stx1, stx2, eaeA, hlyA and saa genes was determined by multiplex-PCR. XbaI-PFGE profiles were analysed using Bionumerics 5.10 software. The strains were O:H serotyped by standard methods, and the ability to ferment sorbitol was tested on SMAC plates and in tubes containing 0.5% sorbitol.

Results: Of all 351 VTEC strains, 153 (43%) were non-O157 serotypes. Of these 16 (10%) belonged to 11 different serotypes that were associated with HUS. These 11 serotypes included O26:H11, O111:H-, O103:H-, O121:H19, O145:H28, O145:H-, O174:H21, O178:H19, R:H4, RH49 and OX182:H25. The median age of the HUS patients was 2.3 years (range 2 weeks–66 years). Of all patients, 8 (50%) were male. All cases were domestically-acquired and sporadic (or family-related) cases. All HUS-related non-O157 strains were sorbitol-fermenting. Among the non-O157 strains, 13 (81%) had stx2 only, two had stx1 only and one strain had both stx1 and stx2 genes. In addition, 10 strains (63%) had eaeA and 14 (88%) had hlyA and one strain had saa gene. All the strains had a unique XbaI-PFGE profile and no clustering was detected within the strains or within one serotype. Interestingly, a rare serotype O78:H- was linked with invasive neonate infection.

Conclusions: The data show that 10% of VTEC non-O157 strains are associated with HUS in Finland which is almost as frequently as within the serotype O157:H7/H-. In the context of a family-related outbreak of VTEC O78:H- in Finland, we found that, in contrast to what has been earlier suggested, VTEC is able also to invade the human bloodstream.

Table 1 Detection of the patients with serotypes and toxin types of EHEC in outbreak by the restaurants

| Group | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | Total |
|-------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|--------|
| O111  | V2 | V2 | V2 | V2 | V2 | V2 | V2 | V2 | V2 | V2 | V2 | V2 | V2 | V2 | V2 | V2 | 9 | 155  |
| O157  | V1 | V1 | V1 | V1 | V1 | V1 | V1 | V1 | V1 | V1 | V1 | V1 | V1 | V1 | V1 | V1 | 2 | 2  |

Toyama 10 14 5 2 1 3 1 22 3 5 1 3 4 49 175

Hokkaido 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1

Fukuoka 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 4

Kanagawa 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 7 181

Total 10 15 5 2 1 3 1 25 3 2 5 1 3 4 101 181
Mixed Etiology Infections Involving Non-O157 Shiga Toxin-Producing Escherichia coli (STEC) – United States, 2001–2010

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Introduction and Objectives: Mixed etiology infections are uncommonly diagnosed, but may provide clues about sources of infections. We summarized mixed non-O157 STEC infections reported to FoodNet from 2001 to 2010.

Material and Methods: We collected data from nine FoodNet sites that conduct active, population-based surveillance for 10 enteric pathogens. A mixed infection was defined as isolation of >1 non-O157 STEC serogroup from the same person within a 7-day period. Exposure and symptom data were collected by patient interview using state-specific report forms. Laboratory data were collected from state public health laboratories and the NationalReference Laboratory for Escherichia Coli, Berlin, Germany.

Results: From 2001 to 2010, 1871 non-O157 STEC infections were reported: 67 (4%) were mixed infections; 60 involved pathogens other than non-O157 STEC and seven involved >1 serogroup of non-O157 STEC. Other pathogens were Campylobacter (42% of 60); Cryptosporidium (38%); STEC O157:H7 (8%); nontyphoidal Salmonella (8%); and Shigella (3%). Of the 67 mixed infections, 21 (31%) were part of outbreaks. Two patients developed HUS, one co-infected with Salmonella serotype Cerro and the other with STEC O157:H7. STEC O111 was isolated in 46% of all mixed infections and 29% of all non-outbreak-associated mixed infections. During the same period, O111 was the third most common serogroup, accounting for 300 (16%) of all 1871 infections. Patients with mixed infections reported bloody stools less often than patients with single-etiologic infections (36% versus 53%). Fifty-one per cent of patients with mixed infections had contact with farm animals and 64% drank well water. By comparison, 29% of persons with single-etiologic non-O157 STEC infections reported visiting a place with farm animals or living on a farm with animals and only 19% of persons in the population survey reported exposure to well water.

Conclusions: Mixed non-O157 STEC infections most commonly involved Campylobacter or Cryptosporidium, pathogens commonly transmitted by water. Exposure data also suggest that drinking well water or having contact with animals may be important exposures for mixed non-O157 STEC infections.
cases. They are responsible for a number of human gastrointestinal diseases, including non-bloody (D) or bloody (BD) diarrhea. In a proportion of individuals, commonly children, these symptoms may be complicated by neurological and renal complication, including hemolytic uremic syndrome (HUS). Most outbreaks and sporadic cases of BD and HUS have been attributed to strains of STEC serotype O157:H7. However, especially in continental Europe but recently also in the USA, the importance of STEC non-O157 as causes of HUS, BD, and other gastrointestinal diseases is being increasingly recognized. Since still few data on clinical human STEC non-O157 infections are available, mainly also in Switzerland a country with a small but disproportionately high travelling population, the aim of this study was (i) to further characterize all STEC non-O157 strains collected by the Swiss National Centre for Enteropathogenic Bacteria from 2000 through 2009 and (ii) to assign the characterized strains to clinical and anamnestic data.

**Material and Methods:** A total of 97 Shiga toxin-producing *E. coli* non-O157 strains from human patients with anamnestic data were collected from 2000 through 2009. STEC isolates were. Fermentation of sorbitol was tested on sorbitol MacConkey agar (SMAC) and PCRs specific for stx1 and stx2 genes, eae, encoding intimin, and ehxA encoding enterohemorrhagic *E. coli* (EHEC) hemolysin were performed.

**Results:** The strains belonged to 40 O:H serotypes with four serotypes (O26:H11/H1; O103:H2; O121:H19; O145:H28/H3) accounting for 46.4% of the strains. Non-bloody diarrhea was experienced by 23.2%, bloody diarrhea by 56.8% of the patients, and 40.0% de- loped hemolytic uremic syndrome (HUS). Serotype O26:H11/H1 was most often associated with HUS. Forty-five (46.4%) strains carried stx2 genes only, 36 strains (37.1%) stx1, and 16 (16.5%) strains both stx1 and stx2. Genes encoding enterohemolysin and intimin were detected in 75.3% and 70.1% of the strains, respectively.

**Conclusions:** Statistical analysis with a multinomial regression and a binary logistic regression showed that strains harbouring stx2 and eae are significantly more likely linked to HUS compared to strains harbouring only one of these virulence factors. interestingly, in our study, from the 29 patients with eae negative isolates, 16 showed bloody diarrhoea and eight developed HUS. These eight HUS cases are linked to a variety of serogroups (O20, O82, O91, O148, O153, O181, ONT) and only five of them harboured the stx2 gene. In our study, 21.1% of the isolates from HUS patients were eae negative. The three eae and stx2 negative strains possess stx1 alone or stx1 and ehxA combined.

**P-012**

**Epidemiology of Shiga toxin Producing Escherichia coli (STEC) in Australia, 2000–2009**

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**Introduction and Objectives:** Reporting of human infection with Shiga toxin-producing *Escherichia coli* (STEC) and clinically diagnosed haemolytic uraemic syndrome (HUS) has been collected Australia-wide since 2000–2001. The purpose of this study was to provide an update on the epidemiology of STEC in Australia using the latest available data.

**Material and Methods:** National and jurisdictional notifications data for STEC infection and HUS, as well as data on serotypes, hospitalizations, mortality and outbreaks were collected from all available sources for analysis and description of Australian STEC epidemiology.

**Results:** For the 10 year period from 2000 to 2009, the annual rate of STEC illness notifications for Australia was 0.4 cases per 100 000 per year. Surveillance practices differ between jurisdictions and annual notification rates varied. The rate for South Australia (SA), the jurisdiction with the most complete surveillance, was 2.4 cases per 100 000 per year and the rates were fairly consistent over the decade. Although diagnostic methods may have varied between laboratories and over time and this may have impacted on the STEC types detected, O serotype was available for 71.2% (504/708) of cases isolates between 2001 and 2009. Of those that were interpretable, 58.0% (225/388) belonged to the O157 serotype, with O111 (13.7%), O26 (11.1%) and O113 (3.6%) the more common non O157 serotypes among STEC isolates typed. Nationally, the proportion of O157 to non O157 strain reported was fairly stable over this period. Between 2001 and 2009 there were 11 STEC outbreaks recorded and these were generally small with a median size of six people. The outbreaks included 100 cases, 30% of whom were hospitalised. Three per cent developed HUS and there were no deaths. The transmission route was reported for 8/11 outbreaks and included person-person (3; O111, OR, O unknown), animal-person (1; O26), waterborne (1; multiple serotypes), foodborne (three with two suspected; all O157) and was unknown for 3 (O86, O55, O unknown). The incidence of total HUS cases in Australia was estimated to be 0.07 per 100 000 per year and 12% HUS cases died between 2000 and 2007. The highest HUS rates were among children <5 years (1.47 cases/100 000 population). The proportion related to STEC infection was not determined. Both STEC and HUS cases showed a similar seasonal distribution.

**Conclusions:** Notifications of STEC infections in Australia have remained fairly steady over the past 10 years. Evidence from outbreaks indicates there are multiple modes of transmission and serotypes involved. Overall, Australia compares favourably to similar countries in terms of the incidence and burden of disease due to STEC and HUS.
and all isolates belonging to other serogroups, was used to estimate virulence gene profiles for the population.

**Results:** A total of 19 402 STEC strains were isolated and characterized during the study period. Ninety-two percent (17 902) expressed or were molecularly matched with known patterns belonging to one of the following O antigens: O157 (13 426), O26 (13 353), O103 (11 335), O111 (10 304), O45 (339), O121 (349) and O145 (268), while 8% (1500) expressed one of 120 other O antigens. Twenty-one serotypes were represented among strains in the seven common serogroups, with the following serotypes predominating within their respective serogroups: O157:H7/NM (100%), O26:H11 (85%), O103:H2 (85%), O111:H8/NM (98%), O45:H2 (97%), O121:H19 (97%) and O145:NM (95%). Two hundred twenty-five serotypes were represented among the second group. The percentages of strains in the common O groups that were positive for stx1/ stx2/ stx1 + stx2, respectively, were as follows: O157: 1/52/47; O26: 96/1/3; O103: 97/<1/2; O111: 69/<1/30; O45: 97/<1/3; O121: <1/97/2; O145: 34/47/19. Over 99% and 98% of strains in this group as a whole were positive for eae and ehxA, respectively. Among strains from the less common serogroups, stx1 alone or in combination with stx2 was present in 38% of strains (571 strains, 74 O groups), and 53% and 77% of strains overall were positive for eae and ehxA, respectively.

**Conclusions:** The combined findings provide a more complete picture of the prevalence and virulence profiles of STEC in the US and show >90% of STEC causing human illness belong to 7 serogroups. A small percentage (2.9%) of diverse strains with the potential to cause severe disease falls outside these groups. Continued surveillance is important to monitor changes in STEC prevalence.

**P-014**

**Geographic Information Systems to Identify Areas at Risk of Sporadic Hemolytic Uremic Syndrome in Buenos Aires City, Argentina**

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**Introduction and Objectives:** In Argentina, the hemolytic uremic syndrome (HUS) caused by Shiga toxin-producing Escherichia coli (STEC) presents frequent sporadic cases. The incidence rate in the year 2007 was 15/100 000 children under 5 years. In this study, we analyzed related factors with the temporal and spatial distribution of sporadic cases of HUS in Buenos Aires City (CABA), Argentina, with the aim to identify areas of major epidemiological risk.

**Material and Methods:** One hundred and six sporadic cases of HUS reported in Buenos Aires City during the period 2005–2009 were evaluated. We recorded gender, age and according to their address each case was georeferenced. We identified percentage of unsatisfied basic needs (% UBN), and the distance from the home to hospitals, fast food places, highways, railways, channeled waterways, low socio-economic settlements and green spaces. Statistical analysis was carried out using difference in proportions test and retrospective space-time analysis scanning for clusters with high rates by the space-time permutation model (SatScan).

**Results:** Significant differences by univariate analysis were found in the proportion of cases in children under 5 years of age, areas with low % UBN, and areas near highways, railways, channeled watercourses, low socio-economic settlements and green spaces. Cases with low % UBN were associated with their proximity to fast food places, railways or green spaces. On multivariate logistic regression analysis, significant risk factors for HUS that remained were low % UBN (OR 24.30; CI 95% 2.04–289.52 P<0.01) and channeled watercourses (OR 16.82; CI 95% 1.48–191.23 P=0.02). The spatial temporal analysis using a permutation model identified 53 clusters of a diameter of <1 km, including 60 cases.

**Conclusions:** Our results allowed identifying areas of risk for HUS with a non-random presentation of the disease and its related factors. The results suggest the existence of ecological niches in the urban area, associated with factors such as the low vulnerability (% UBN) and proximity to channeled watercourses, which determine the highest probability for STEC, the most common etiologic agent to circulate. The clusters identified might have common sources of infection, such as fast food places or the presence of reservoirs which, in a limited radius, could contaminate stored food. Among them, synanthropic animals, whose habitat overlaps with railways, channeled waterways and green spaces should be considered.

**P-015**

**Family Outbreak Caused by Shiga Toxin 2-producing Escherichia coli O26:H11 with a Fatal Case of Hemolytic-Uremic Syndrome**

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**Introduction and Objectives:** Similar to other countries, enterohemorrhagic Escherichia coli (EHEC) cause severe human diseases also in the Czech Republic. During 2006–2011, the National Reference laboratory for E. coli and Shigella of NIPH in Prague received 27 EHEC strains isolated from patients with diarrhea-associated hemolytic-uremic syndrome (HUS) or bloody or non-bloody diarrhea without HUS. EHEC O26:H11/NM was most frequent serotype in this collection. Here we present characteristics of four EHEC O26:H11 isolates from members of one family. Two year-old girl died of a severe HUS.

**Material and Methods:** EHEC O26:H11 strains were isolated in June 2009 from the stools of a 2-year-old girl with HUS, and her 8-year-old twin sisters and their grandmother who were all asymptomatic. The isolates were confirmed as E. coli (API 20E; bioMerieux), and serotyped, tested for sorbitol fermentation, beta-D-glucuronidase activity, tellurite resistance, production of Shiga toxins 1 and 2, and EHEC hemolysin phenotype. The stx1 and stx2, eae, and plasmid virulence genes (EHEC-hlyA, etpD, katP, espP) were detected by PCR. stx genes and blF encoding the flagellin subunit of the H antigen were subtyped using restriction fragment length polymorphism approaches. Macrorestriction analysis using PFGE was applied to verify epidemiological relatedness of the isolates.

**Results:** The isolate from the HUS case, and all three isolates from the family members had the same phenotypes and genotypes. They belonged to serotype O26:H11, were sorbitol-positive, beta-D-glucuronidase-positive, and tellurite resistant, and produced Shiga toxin 2 and EHEC hemolysin. All contained stx1 (but not stx2), eae β, and combination of plasmid genes typical for the new O26 clone previously identified in Germany (i.e. presence of EHEC-hlyA and etpD, and absence of katP and espP). They shared identical PFGE patterns demonstrating their epidemiological relationship.

**Conclusions:** EHEC O26:H11 which produced Shiga toxin 2, was the etiological agent of a family outbreak including a fatal HUS case. This demonstrates potential of Shiga toxin 2-producing EHEC O26 to cause outbreaks and severe disease.
P-016
Prolonged Fecal Shedding of Shiga Toxin-Producing Escherichia coli in Children Attending Day-Care Centers in Argentina
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Introduction and Objectives: Shiga toxin-producing Escherichia coli (STEC) is a major cause of serious outbreaks and sporadic cases of hemorrhagic colitis and hemolytic uremic syndrome (HUS) in humans. Person-to-person transmission is well documented in day-care centers because of the low infective dose of this pathogen. The prolonged and intermittent shedding of STEC could be another important risk factor considering it increases the likelihood of dissemination. The purpose of this study was to describe the long shedding of STEC during three outbreaks that occurred among children attending day-care centers in Argentina.

Material and Methods: Six children involved in three outbreaks that had occurred in different locations in Argentina between June 2008 and June 2011 were studied. After the first STEC detection, the children were followed up by fecal sampling with a 48–72 h interval until confirmation of two consecutive negative samples. The fecal samples were sent to the National Reference Laboratory, and processed by standardized protocols. A total of 40 samples were studied: seven from one child (1st outbreak), 23 from three children (2nd outbreak), and 10 from two children (3rd outbreak).

Results: 1st outbreak: in June 2008, a child attending a day-care center in Buenos Aires City had bloody diarrhea associated with STEC O157:H7, stx1/stx2c, eae, ehxA. The pathogen was detected in four consecutive samples during 52 days. 2nd outbreak: from April to June 2010, an outbreak occurred at a day-care center in Bariloche City. STEC O121:H19 stx2, eae, ehxA was isolated from an HUS case and one asymptomatic contact, during 37 and 17 days, respectively. In another asymptomatic contact the excretion was intermittent during 52 days. 3rd outbreak: from April to June 2011, one case of non-bloodly diarrhea and one case of bloody diarrhea occurred at a day-care center in Buenos Aires City associated with STEC O145:NM, stx2, eae, ehxA. The shedding was 50 and 26 days, respectively.

Conclusions: In this study all cases and contacts showed prolonged shedding. In one asymptomatic contact, an intermittent shedding was demonstrated. It is important to improve the sanitary controls in day-care centers to prevent STEC dissemination in order to reduce the risk of an outbreak.

P-017
Outbreaks of Shiga Toxin-Producing Escherichia coli in Argentina, 2007–2011
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Introduction and Objectives: In Argentina, many outbreaks of non-bloody (NBD) and bloody diarrhea (BD), and hemolytic uremic syndrome (HUS) associated with O157 and non-O157 STEC infections have been identified through the national surveillance system. The purpose of this study was to describe STEC-associated outbreaks that occurred in the community, families, and kindergartens between 2007 and 2011.

Material and Methods: According to the surveillance system, when an HUS or STEC-associated diarrhea case is identified, an epidemiologic investigation is triggered, including the study of household and institutional contacts. Further, STEC strains isolated are characterized by biochemical tests, serotyping, genotyping, and pulsed-field gel electrophoresis (PFGE) using the 24-h PulseNet standardized protocol.

Results: A total of 333 individuals were involved in 12 outbreaks that occurred in the central and southern regions of the country, one in the community, 5 in families, and 6 in kindergartens. HUS cases occurred in 10/12 (83%) outbreaks, and they were identified as index cases in 7/12 (58%). In different kindergarten events, three HUS cases died. During the epidemiological investigation, 13 HUS, 8 BD, and 11 NBD STEC-positive cases were confirmed by the laboratory. In addition, 24 STEC-infected asymptomatic contacts were detected. In 9/12 (75%) outbreaks. In 8/12 (66.7%) outbreaks, STEC O157:H7 stx1/stx2c/ehxA in the community outbreak, O145:NM stx2c/ehxA and O121:H19 stx2c/ehxA in two kindergarten outbreaks, and ON9:H19 stx2c in one family outbreak. In a family outbreak, bovine meat and fresh sausage were identified as the source. Person-to-person transmission was the probable route in most of the outbreaks. XbaI-PFGE patterns were compared from the PN databases of the USA, Japan, and Argentina.

Conclusions: Family and kindergarten outbreaks associated with STEC infection are often detected in Argentina. It is necessary to enhance active surveillance for STEC-diseases including specific molecular laboratory activities to identify the source, reduce the possibility of transmission and prevent new cases. In addition, the knowledge of transmission routes and vehicles will allow professional health workers, teachers and parents to be educated on how to reduce risky behaviors, which can decrease their risk for STEC infection.

P-018
International Clones of STEC O157 Studied by PFGE and Virulence Profile in Three PulseNet Countries: USA, Japan and Argentina
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Introduction and Objectives: Different surveillance strategies for STEC O157 infectious disease are performed worldwide. PulseNet (PN) monitors sporadic cases to detect and investigate clusters caused by STEC from its Pulsed-Field Gel Electrophoresis (PFGE) patterns. In this study, we analyzed the circulation of STEC O157 XbaI patterns appearing among the 10 most common patterns in at least one of three countries participating in PN International. PFGE patterns were compared from the PN databases of the USA, Japan, and Argentina during 2006–2011.

Material and Methods: XbaI-PFGE patterns of human isolates were generated using the PN standard protocol for STEC O157. Data from 14 711 isolates (2877 patterns) were available from USA as were data on 10 964 isolates (4610) from Japan, and 772 isolates...
P-019
Surveillance of Pediatric Hemolytic Uremic Syndrome (HUS) in Italy, 1988–2011

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Introduction and Objectives: HUS is a common cause of acute renal failure in children. In Italy, a National Registry of pediatric HUS was established since 1988 by the Italian Pediatric Nephrology Society, in cooperation with the National Reference Laboratory for E. coli. The aims were to carry out surveillance of HUS, to estimate the burden of the disease in the population and to monitor the associated infections with to Verocytotoxin (VT)-producing E. coli (VTEC).

Material and Methods: The population under surveillance included children <15 years of age and a case of HUS was defined as a patient with acute renal failure and microangiopathic hemolytic anemia (Hb < 10 g/L) or thrombocytopenia Plt < 100 000 mL). Clinical and epidemiological information were collected, together with stool and blood samples. Laboratory diagnosis of VTEC infection was based on the isolation of VTEC strains, the detection of free VT in stools and the detection of serum antibodies to the lipopolysaccharide (LPS) of the most important serogroups. A subset of patients was also examined for the presence of VT in their circulating polymorphonuclear cells (PMN).

Results: Up to August 2011, 742 cases were identified, accounting for a mean annual incidence of 0.38 × 10⁻⁵ (0.91 × 10⁻⁵ in children of 0–4 years of age). The incidence rate was higher in the northern regions of Italy. Several epidemic outbreaks, involving a number of cases ranging from 15 to 2 cases, were observed. The mean age of the patients was 38 months (median 24), and most of them were in the age group 0–4 years. Prodromal diarrhea was observed in 563 cases (82.7%): 315 (55.7%) had bloody diarrhea and 250 (44.4%) watery diarrhea. Clinical samples were obtained from 508 cases and the laboratory assays provided evidence of VTEC infection for 351 of them (69%). The VTEC serogroups involved were O157 (36.3%), O26 (29.6%), O145 (11.3%), O111 (11.1%), and O103 (7.1%). Other 11 serogroups, including O121 and O55, accounted for 5.0% of cases. Cases associated with VTEC non-O157 infections, particularly O26 and O145, increased over time, and in the decade 1998–2007 outnumbered those associated with VTEC O157. Patients with VTEC non-O157 infections were younger (median: 22 months) than those with VTEC O157 (median: 32 months). A more clear summer pattern was observed for VTEC O157 infections. Atypical HUS cases, for whom no prodromal diarrhea nor diagnosis of VTEC infection were reported, were 49, representing the 9.2% of the 531 cases with information on both characteristics available.

Conclusions: Surveillance data suggest that the incidence of HUS in Italy is lower than those reported for other industrialized countries and that VTEC non-O157 have an important role in its etiology. This highlights the need for a comprehensive laboratory approach to the diagnosis of VTEC infection.

P-020
Persistence of Enterohaemorrhagic Escherichia coli (EHEC) in Patients

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Introduction and Objectives: The pathovar of vero toxin-producing Escherichia coli (EHEC) is already known as an enteric pathogen for men. In most cases the symptoms pass off a few days after infection. But does this mean that the pathogen has disappeared from its host?

Material and Methods: In our lab stool specimen collected by the health-office and isolates from patients are investigated for EHEC by ELISA and stx-PCR. Isolates were further characterized by serotyping. As some patients, especially workers in canteens or communal feedings, have to be controlled by law for the absence of these bacteria, we were able to follow its persistence in individuals over months. Patients following three requirements were selected: (i) More than one sample (stool or strain) was positive for EHEC; (ii) Both of two samples were not taken or sent the same day; (iii) Only samples from one patient were included that exhibited EHEC isolates presenting identical antigenic formulae, but also developments to O rough forms were included. Patients were defined as identical, when family name, first name, and date of birth or address were the same. The respective duration of persistence was defined as the time span between the first and the last date of an incoming positive sample. Culture media for growth as well as diagnostic sera for agglutination of EHEC were home made.

Results: The first data pool collected from 1999 to 2010 comprises 106 patients, of which 94 lived in Hamburg, and 12 at four other locations in Germany. The mean age of the patient pool was 32, the median 33 years. The age spectrum included <1 and up to 81 years. Of the 106 patients 69 were female and 37 male. Among children up
to 14 years male patients (17 of 26) were dominant. Among older patients the females (60 among 80) dominated. For this pool calculations revealed 29 days as the median and 69 days as the mean duration of EHEC bacteria present in the pool patients. About one half of them (n = 52) were positive for EHEC for up to four weeks. However, 54 patients exhibited a prolonged persistence of the pathogen for up to 383 days. The O-types O91, O128 and O146 were predominantly found among the long-term persistent E. coli. A second pool analyzed since May 2011 included 126 patients suffering from the O104:H4 outbreak strain, of which only two were from outside Hamburg. The mean age of this patient pool was 38, the median 35 years. The age spectrum included <1 and up to 84 years. Of the 126 patients 86 were female and 40 male. About one half of them (n = 67) were positive for EHEC for up to four weeks, and 59 patients exhibited a prolonged persistence of the pathogen. Up to now both pools only differ in mean age (32 versus 38).

Conclusions: The results make obvious that an EHEC patient could be a long lasting source for the pathogen.

P-022
Search for an Association Between Polymorphisms of CX3CR1 Gene and Typical Hemolytic Uremic Syndrome in Argentine Children
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Introduction and Objectives: Hemolytic uremic syndrome (HUS) is a vascular disease characterized by microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure. Its typical form is a systemic complication of Shiga toxin-producing Escherichia coli infections that occurs in 10% of infected children. We have found that HUS patients present a significant decrease in circulating leukocytes expressing the CX3CR1, which correlated with poor prognosis. In addition, CX3CR1+ leukocytes were detected in renal biopsies of HUS patients. Two common single-nucleotide polymorphisms (SNPs), which cause amino acid changes in codons 249 and 280 of CX3CR1 (V249I and T280M), have been described. These two polymorphisms are in strong linkage disequilibrium, forming a common I249-M280 haplotype which have been associated with a reduced risk of acute coronary events and atherosclerosis. The aim of our work was to study the prevalence of CX3CR1 polymorphisms in Argentine children and investigate the association with HUS occurrence in a case-control cohort.

Material and Methods: DNA extraction from urine samples of 87 HUS and 132 age-matched healthy controls (HC) was performed using a DNA extraction kit. The SNPs of CX3CR1 gene were studied using a DNA extraction kit. The SNPs of CX3CR1 gene were screened by PCR-RFLP technique. Allelic frequencies were calculated by gene counting. Hardy–Weinberg (H-W) equilibrium was tested by a χ² test. Unadjusted univariate analyses of the relationship between CX3CR1 genotypes and HUS was performed by cross-tabulation and results were expressed as odds ratios (ORs) with 95% confidence intervals (CI)s. Significance of the associations was evaluated based on χ² tests.

Results: The genotype distribution of each individual allele was consistent with the H-W prediction in HC. However, we observed a deviation for V249I allele in HUS. Moreover, there was no significant difference in genotype frequencies between HUS and HC. The unadjusted ORs associated with I249 (VI+II versus VV genotype) and M280 (TM + MM versus TT genotype) were 0.82 (95% CI, 0.48–1.42, P = 0.49) and 1.04 (95% CI, 0.57–1.89, P = 1), respectively. The frequencies of combined genotypes and haplotypes were similar between HUS and HC. The OR for VI-TT versus VV-TT was 0.46 (95% CI, 0.19–1.12, P = 0.10) and for VI-TM versus VV-TT was 0.97 (95% CI, 0.47–2.01, P = 1). However, VI-TT genotype was less frequent in HUS compared to HC (9% versus 18%) showing a tendency to correlate with reduced risk of HUS development.

Conclusions: This is the first study to characterize the CX3CR1 gene polymorphisms in Argentine children and HUS patients. There were no differences in the allele or genotype frequencies between HUS and HC. Although we observed a lower frequency of VI-TT genotype in HUS patients, we could not find an association between CX3CR1 V249I and T280M polymorphisms and HUS development in Argentine children.

P-023
Rapid Detection, Identification and Communication of Swedish Cases in the German STEC O104:H4 Outbreak – a Description of the Swedish Outbreak
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Introduction and Objectives: In the middle of May 2011 Sweden investigated a cluster of patients suffering from bloody diarrhoea and HUS following a short trip in the north of Germany. On 22 May, an EWRS alert on an observed increase of STEC diarrhoea and HUS cases was posted by Germany. Immediately after the alert, it became clear that this was an unusual international event and a Swedish outbreak investigation was started. The objectives were to identify all cases and to trace their possible source of infection to prevent new cases and to assist Germany in stopping the outbreak.

Material and Methods: Infection with STEC is notifiable in Sweden and cases are reported daily from both clinicians and laboratories in a web-based surveillance system where regional and national departments receive the cases simultaneously. During the outbreak, clinicians and laboratories were urged to enhance surveillance and sampling of suspected STEC patients and to send isolates for typing. All cases were interviewed regionally regarding travel facts, hotel stays and where and what they had eaten in Germany. A cohort study was performed on the primary cluster of cases. All information was continuously communicated to the German authorities. Information to the Swedish public, the media and to the health care system was communicated via the Swedish Institute for Communicable Disease Control (SMI) website.

Results: As of 4 July, when the outbreak was formally declared over, 53 Swedish cases had been identified including 18 (34%) HUS cases and one death. More women were reported, especially evident for the HUS cases. All cases were above 20 years of age. Two days after information on the outbreak strain was available the first confirmed outbreak case was identified using PCR-based serotyping of O104 and PFGE analysis in SMI. In total, aggregative STEC O104 was isolated from 48 out of 53 cases. Few secondary cases were reported (three of which one HUS) and one primary case was reported as domestic. All other cases had been travelling in the north of Germany (median incubation period of 7 days (2–18)). Several Swedish clusters were early identified in German hotels and restaurants which...

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later were figuring in German cohort studies. The Swedish cohort study unfortunately did not show any significant risk associated with any food item.

**Conclusions:** Sweden reported the highest number of cases outside Germany. The unusual epidemiological characteristics of the cases were similar to the observations in Germany. Sweden has a web-based surveillance system that enables regional and national levels to share information simultaneously. This together with a microbiological preparedness for characterisation of new strains enabled a rapid response to the German outbreak alert and could early point to certain hotels and restaurants for further investigation by the German authorities.
Clonal Relatedness of Atypical EPEC O157:H16 Strains Isolated from Clinical and Environmental Sources from Several Countries Worldwide

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Introduction and Objectives: Enteropathogenic Escherichia coli (EPEC) is a common cause of infantile diarrhea in developing countries. Typical EPEC carry the locus for enterocyte effacement (LEE) pathogenicity island and the EPEC adherence factor (EAF) plasmid and induces attaching and effacing lesions on the intestinal epithelium. Atypical EPEC (aEPEC) strains have LEE but not the EAF plasmid and have also been associated with diarrhea in children. aEPEC strains are reported to be phylogenetically diverse, but, an analysis of O157:H16 aEPEC strains from water in the U.S. and meats in France showed these to have identical sequence type (ST-171) and similar PFGE profiles. To determine if the same strain had disseminated to both countries or whether the aEPEC O157:H16 is a conserved clonal group that exists worldwide, we tested other O157:H16 strains from various countries and analyzed their virulence profiles and clonal relatedness.

Material and Methods: The 45 O157:H16 strains consisted of 21 from Germany (clinical), 11 from Argentina (animals), 10 from The Netherlands (animal) and 3 from Norway (clinical). All isolates were tested by PCR for the intimin-encoding eae gene that resides on the EAF plasmid and have also been associated with diarrhea in children. aEPEC strains are reported to be phylogenetically diverse, but, an analysis of O157:H16 aEPEC strains from water in the U.S. and meats in France showed these to have identical sequence type (ST-171) and similar PFGE profiles. To determine if the same strain had disseminated to both countries or whether the aEPEC O157:H16 is a conserved clonal group that exists worldwide, we tested other O157:H16 strains from various countries and analyzed their virulence profiles and clonal relatedness by MLST.

Results: Of the 45 strains, 39 had eae and, except for two German strains that had b-eae, carried the eae allele. All 39 eae (+) strains were aEPEC, as none had the bfpA gene, suggesting the absence of EAF plasmid. The six eae (-) strains had distinct PFGE profiles and clustered apart from the eae (+) strains, which clustered amongst each other and shared profile similarities. When compared to the U.S. and French O157:H16 strains examined earlier, most of the eae (+) O157:H16 strains had very similar PFGE profiles with a few U.S. isolates having nearly identical profiles to some of the German isolates. MLST showed that the eae (-) O157:H16 strains were ST-344, excepting one strain that was a ST-83 variant. In contrast, all eae (+) O157:H16 strains, regardless of the eae allele carried, were ST-171, a finding that is consistent with the results obtained from the U.S. and French O157:H16 strains examined earlier.

Conclusions: Results show that the O157:H16 serotype is phylogenetically diverse and comprised of strains with different ST and PFGE profiles. But, the aEPEC strains within the O157:H16 serotype, regardless of the eae allele carried, is a fairly homologous group of ST-171 strains having similar PFGE profiles. The fact that these strains were isolated from various clinical and environmental sources from six different countries worldwide strongly suggests that the aEPEC strains of O157:H16 serotype are a highly conserved clonal group.

Evaluation of an Immuno-Chromatographic Detection System for Shiga toxins and the E. coli O157 Antigen

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Introduction and Objectives: Contaminated foodstuff is the major vehicle of transmission for Shiga Toxin producing Escherichia coli (STEC) strains to humans. A rapid detection of STEC from sample material is important for food safety. The detection of Shiga Toxins (Stx) or stx-genes is the only way to identify all members of STEC. Commercialized standardized Stx detection kits are widely used in diagnostic laboratories. Here, we have evaluated an immuno-chromatographic lateral-flow test for the detection of Stx and the E. coli O157 antigen from pure cultures and from EHEC spiked salad samples.

Material and Methods: The ‘RIDA Quick’ test (RIDA® QUICK Verotoxin/O157 Combi; R-biopharm) is an immuno-chromatographic system for detection of Stx and the O157 antigen. It is a single-step lateral-flow test, where specific antibodies against target antigens are attached to red (Stx-specific) or green (O157-specific) latex particles. Supernatants of bacteria and food samples grown in mTSB + Mitomycin were used for the test. The colour reaction is visible after 15 min. The Vero cell test was used as a gold-standard for Stx activity and the Ridascreen® Verotoxin enzyme immunosassay (R-biopharm) was used for comparison.

Results: The RIDA Quick test was evaluated with reference strains for all known variants of Stx1 and Stx2. All 20 strains of the Stx1 family (Stx1a (n = 2), Stx1a (9), Stx1c (5) and Stx1d (4)) were detected. Of 55 strains of the Stx2 family all Stx2a (12) and Stx2c (6) strains were detected. Some of the Stx2b (8/9 positive) Stx2d-activatable (5/7 positive), Stx2e (3/11) and Stx2g (1/4) strains were not detected as well as all Stx2f strains (0/6 positive). False positive reactions were not found. The specificity for the E. coli O157 antigen was tested with 134 strains (45 E. coli O-groups) including 17 O157 strains. The RIDA Quick assay detected all E. coli O157 strains tested. False positive reactions were not observed. The sensitivity of the RIDA Quick test for detection of Stx and the O157 antigen was analyzed with 25 g salad samples spiked with different amounts (<10, 10–100, 100–1000 CFU) of EHEC O26, O103, O111, O145 and O157 strains. All spiked salad samples gave positive reactions for Stx and O157, respectively. No false positive reaction was found.

Conclusions: The RIDA Quick detected all EHEC-associated Stx-types (Stx1a, Stx2a and Stx2c) and other Stx2 variants except Stx2f. Negative results obtained for some Stx2b, Stx2d, Stx2e and Stx2g strains are probably due to the poor Stx production, which is below the detectable level for the assay. For Stx, the results obtained with the Rida Screen EIA were identical to those obtained with the RIDA Quick Test. Both tests are from the same producer, it is possible that the same Stx-antibodies are used in both assays. First results with EHEC inoculated salad samples indicate that the RIDA Quick assay is suitable for EHEC screening in food.
Identification and Validation of Specific Molecular Targets for Detection of Escherichia coli O104:H4 Causing a Large Outbreak of Hemolytic Uremic Syndrome in Germany in May 2011

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Introduction and Objectives: During spring 2011, Europe faced its largest STEC outbreak involving an emerging pathogenic, E. coli O104:H4, and presenting a new virulence pattern, combining some of the virulence factors found in EHEC (Enterohemorrhagic E. coli) and EAggEC (EnteroAggregative E. coli) strains. Although traditional culture and phenotypic tests can identify the outbreak strain, rapid molecular testing is useful for timely diagnosis.

Material and Methods: Three hundred and five E. coli strains, including the 186 known O-serogroups and 53 H-types were investigated to evaluate the specificity of qPCR assays targeting the O104 antigen (rbE078, wz078, wz078), the H4 flagellar antigen (flaC4), the regulator aggR as a global regulator of the EAggEC virulence factors, and a non-coding region that can be used as a single PCR target.

Results: The PCR assays based on the O104 and H4 sequences showed a high specificity and concordance with serology when testing a collection of 305 E. coli strains, including the 186 known O-serogroups and 53 H-types. The PCR assays targeting the O104 sequences detected all E. coli strains of serotypes O104:H2, O104:H4, O104:H7, O104:H11, O104:H12, and O104:H21, giving only cross-reactions with E. coli strains carrying a K9 capsular antigen, such as O8:k9:H10, O9:k9:H1 and O9:k9:H12. The gene aggR was detected in all the 51 O104:H4 epidemic and non-epidemic strains, the EAggEC reference strain 17-2 (serotype O3:H2) and some STEC and non-STEC strains of serotype O111:H10. While multi-target PCR assays combining multiple pairs of primers could be designed for testing e.g., genes coding for Shiga toxin (Stx), O104 antigen, H4 antigen, and AggR, none of the targets is unique to the outbreak strain and an isolate is identified only if PCR results for all loci are positive. Hence, such assays are limited to cultured isolates and have limited use in uncultured clinical, food, or environmental samples.

Based on genome analysis of the O104:H4 epidemic strain we identify a non-coding region that can be used as a single PCR target for identification and detection of the O104:H4 outbreak strain. Using this specific target, we designed a robust qPCR assay capable of detecting 51 O104:H4 isolates related to the outbreak occurring in May 2011 and to one O104:H4 clinical isolate reported in 2001. The E. coli O104 strains having other H-types than H4 and the E. coli strains carrying a K9 capsular antigen tested all negative. In final, among the 305 E. coli strains that include the 186 known O-serogroups and 53 H-types, only two isolates of serotypes O11:H2 and O43:H2 were cross reacting with the primers and probes designed in this sequence.

Conclusions: These multi-target qPCR assays proved to be specific for identifying E. coli O104:H4 causing a large outbreak of HUS in Germany in May 2011.

P-030
Proposal for a Renewed Consensus Nomenclature of Vero/Shiga Toxin-Producing Escherichia coli
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Introduction and Objectives: The term Vero/Shiga-toxigenic Escherichia coli (VTEC/STEC) refers to any E. coli producing a Vero/ Shiga toxin (VT/Stx) (Konowalchuk et al., 1977; O’Brien et al., 1982). The clinical significance of VT/Stx was established in 1983

United States Public Health Guidance for the Isolation and Characterization of Shiga Toxin-Producing Escherichia coli from Clinical Samples
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Poster Presentations

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with the demonstration of their role in haemorrhagic colitis (HC) and haemolytic-uremic syndrome (HUS) caused by O157:H7 *E. coli* in humans (Karmali et al., 1983). But O157:H7 STEC had already been named 'enterohaemorrhagic *E. coli* (EHEC)', a name that survived the description of the production of VT/Stx and of their other main virulence-associated property i.e. the attaching/effacing (AE) lesion in the gut. Besides O157:H7, several other EHEC serotypes are responsible for uncomplicated bloody or non-bloody diarrhoea and HUS in humans. If some authors prefer to limit the use of 'EHEC' to the O157:H7 strains (and name the others strains 'atyphical EHEC' or 'EHEC-like'), others extend the name EHEC either to all serotypes that can cause HC and/or HUS, or to all VT/Stx+ve *E. coli*. To add to the confusion, some VTEC/STEC not producing AE lesion are also responsible for HUS while others form groups of animal-specific pathogens like those causing oedema disease in piglets. Those VTEC/STEC differ by their combinations of virulence-associated properties others than VT/Stx and new combinations may always arise like the recent enteroaggregative (EAE) O104:H4 VTEC/STEC in Germany. A simplification of the situation is urgently needed and we propose a renewal of the nomenclature of VT-producing *E. coli*.

**Material and Methods:** The first base would be to keep the name VTEC/STEC that refers to the production of VT/Stx. The second base would be to add a suffix to qualify the other virulence-associated properties: AE-VTEC/STEC for those producing AE lesion (like O157:H7 EHEC), Saa-VTEC/STEC for those producing the Saa adhesin, Agg-VTEC/STEC for the enterohaemorrhagic strains (like the O104:H4), F18-VTEC/STEC for those causing oedema disease in piglets, etc. In addition we propose to entirely discard the name EHEC that refers to a clinical condition and is confusing as bloody diarrhoea is not always prominent in the clinical presentation.

**Conclusions:** Such nomenclature also opens the possibility to include new combinations of virulence-associated properties that may arise in future. Moreover these 'new' names can easily be combined the seropathotype grouping scheme of Karmali and collaborators (Karmali et al., 2003): strains of seropathotype groups A and B are AE-VTEC; those of group C are either AE-VTEC or Saa-VTEC or the newcomer O104:H4 Agg-VTEC, etc.

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outbreak situations. ‘Gold standard’ is an agglutination procedure for O- and H-serotyping which, however, is very time-consuming [1]. The interpretation of results is challenging due to possible cross-reactions or masking of capsular antigens. Microarray technology circumvents these problems and allows serotyping of E. coli strains in a fast and reliable manner [2].

**Material and Methods:** For this study we used customer designed oligonucleotide based microtube DNA microarrays (Identibac Array-Tube™). The microarrays contained oligonucleotide DNA probes for the 24 epidemiologically most relevant O-specific genes as well as for 47 out of the 53 different H-antigen genes of E. coli. Extracted bacterial DNA was amplified and comcomitantly labelled with biotin in a linear multiplex PCR reaction. Next, the biotin labelled linear PCR products were hybridized to the corresponding probes. Horse-radish peroxidase (HRP)-streptavidin and SeramunGrün® were added and the coloured precipitate was detected with an Array-Tube™ Reader and further analyzed with the supplied software.

**Results:** First we evaluated the microarray technology with 18 well-characterized reference E. coli strains. The arrays showed 100% accordance with conventional O- and H-serotyping. Next, we genetically retyped seven H-non-typeable, 15 H-rough and eight H-nomotile E. coli strains sent to the National Reference Center for Escherichia coli. Different H-serotypes were determined for all of these 30 strains. The corresponding O-serotypes could not be identified for all of them, due to the fact that only the most relevant 24 O-specific genes are spotted on the array.

**Conclusions:** The microarray technology is a fast and reliable alternative to the conventional E. coli serotyping method. It allows identification of the H-serotypes and the 24 most relevant O-serotypes of E. coli strains and moreover reveals additional information about non-typeable strains, non-motile strains and strains showing the rough phenotype.

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**P-034**

**Contribution of Real-Time PCR Assays to the Detection and Quantification of the Main Pathogenic STEC in Foods**

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**Introduction and Objectives:** Enterohaemorrhagic E. coli (EAHEC) O104 Strains of Different Origin and Sources

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**Introduction and Objectives:** Enterohaemorrhagic E. coli (EAHEC) O104:H4 have caused a large outbreak in May 2011 in Germany with 3842 patients and 855 HUS-cases. EAHEC O104:H4 are known since 2001 as agents of diarrhoea and HUS in countries in Europe and Asia. Besides EAHEC O104:H4, STEC O104:H2, O104:H7 and O104:H21 were implicated in outbreaks and sporadic infections of humans in different countries. Here, we have analysed 55 E. coli O104 strains from humans, animals and food originating from different countries in Asia, Europe and North America for their genetic similarity by PFGE and for their virulence genes with a PCR microarray. The results indicate that two different clones of EAHEC O104:H4 have emerged and are spread over geographically distant places.

**Material and Methods:** Fifty-three E. coli O104 strains from human, animal and food were investigated. Thirty-five of these were EAHEC O104:H4 originating from humans and food in Germany, Norway, the Republic of Georgia and the USA. The remaining strains were O104:H2 (n = 2), O104:H7 (n = 4), O104:H12...
(n = 1) and O104:H21 (n = 11). Investigation of genotypes was done by pulsed-field gel electrophoresis (PGFE) with XbaI digested total bacterial DNA (CDC PulseNet protocol). The presence of 59 genes coding for E. coli O and H antigens, LEE and non-LEE effectors, adhesins, antimicrobial resistance and genes located on EHEC- and EAEC-virulence plasmids was investigated with a real-time PCR microarray. Subtyping of stxl and stx2 genes was performed by RFLP-analysis of stx-specific PCR products.

Results: Production of Stx and presence of stx-genes were found in all O104 strains except for one O104:H2 and one O104:H12 strain. All O104:H4 strains were positive for the stx2 gene; other virulence genes of classical EHEC (LEE and nle-genes, EHEC-plasmid) were not found. O104:H21 strains were positive for stx1 and stx2, enterohaemolysin (exhA) but negative for LEE and nle-genes. STEC O104:H2 and O104:H7 carried only stx1c and stx2b genes. All O104:H4 strains carried virulence genes characteristic of EAEc (aatA, aggA or agg3A, aggR, aap, set1 and pic). EAHEC O104:H4 divided into two genetic clusters with 85.1% similarity. Cluster 1 strains (agg3A, astA) were isolated from HUS cases in 2001 in Germany and in 2005/2006 in Norway from patients with bloody diarrhoea. Cluster 2 strains (aggA, negative for astA and positive for ESBL) were isolated from humans and food related with the EAHEC O104:H4 outbreak in Germany in 2011. O104 strains expressing other H-antigens found were genetically unrelated to the O104:H4 strains (similarity 64.9–73.3%).

Conclusions: The findings are indicating that two genetically related and highly virulent types of EAHEC O104:H4 have spread over large geographic regions. EAHEC O104:H4 were not associated with animals and food of animal origin. In contrast, STEC O104:H21 STEC was frequently associated with cattle and bovine products.

P-036
Public Health Significance of E. coli O26 Isolated from Foodstuffs: Toward Genetic Predictors of Their Virulence
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Introduction and Objectives: Enterohemorrhagic E. coli (EHEC) are zoonotic pathogens responsible of hemorrhagic colitis and the life-threatening hemolytic-uremic syndrome in humans. Infections are mainly acquired through consumption of contaminated foodstuffs. Only a limited number of serogroups have been identified from severe diseases including O26 which is increasingly reported worldwide. Shiga-toxins (Stx) are the main virulence factors of EHEC and are encoded by bacteriophages. It is known that EHEC O26 can lose stx genes both in vitro and in vivo and that some stx-negative E. coli O26 strains can acquire Stx phages in vitro. In foodstuffs, it remains impossible to differentiate non pathogenic E. coli O26 strains that cannot be lysogenized with Stx phages from EHEC derivatives or from strains that might be converted to real pathogens upon lysogenization.

Material and Methods: A strategy based on the comparison of the virulome and the complete genetic profiles of 123 E. coli O26 strains isolated from both humans and foodstuffs (mainly dairy products) was carried out. For this, 59 virulence associated genes were screened both in vivo and in vitro. To search for VTEC. We investigated the incidence and virulence determinants of VTEC infections in a multicenter study in Brussels-Capital Region (BCR) (Belgium) from April 2008 to October 2010.

Material and Methods: Stools were collected by seven hospital laboratories for microbiology in BCR. Colony sweeps from SMAC/CT-MAC were screened for vtx1, vtx2, and vtx2f by multiplex PCR. E. coli isolates were biochemically confirmed, serotyped, and investigated for virulence markers. Pulsed-field gel electrophoresis (PGFE) was used to assess their molecular relatedness.

Results: Overall, 206 (1.40%) of 14 705 stools were vtx+ by PCR, with the highest incidence detected in patients with HUS (35.3%), a history of BD (5.15%), stools with macroscopic blood (1.86%), and in young children (1.89%). VTEC infections, O157 in particular, were more common during the summer months (P < 0.0001). VTEC were isolated from 140 (67.9%) of 206 vtx+ stools. One sample yielded two different serotypes, thus, 141 isolates could be characterized. Sixty different O:H serotypes harboring 85 different virulence profiles were identified. Most isolates (75.9%) belonged to non-O157 serogroups. Fifty-nine (41.8%) isolates were positive for vtx1, 55 (39.0%) for vtx2 (including 18 vtx2f), and 27 (19.1%) for both vtx1 and vtx2. Remarkably, vtx2f was the second-most common subtype detected in non-O157 isolates. PGFE revealed wide genetic diversity, however, small clusters of O157, O26, and O63:H6 could have been part of unidentified outbreaks.

Conclusions: Our data show that VTEC of many serotypes and carrying heterogeneous virulence profiles were associated with human
disease. Clinical laboratories could consider screening of selected samples, if not all, for VTEC O157 and non-O157 using selective media and a method detecting VT of vtx genes.

P-040
Multi locus Sequence Types (MLST) of O113:H21 Shiga Toxin-Producing and stx-Negative Escherichia coli Strains in Brazil

Introduction and Objectives: Shiga toxin-producing Escherichia coli strains (STEC), including those belonging to serotype O113:H21, are important human pathogens associated with different clinical manifestations as non-complicated diarrhea, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). In Brazil O113:H21 STEC is highly prevalent among ruminant animals which constitute a natural reservoir for such strains. However, in our settings human infections due to this serotype have been associated to E. coli strains lacking stx genes. Genetic virulence profiling of human and non-human O113:H21 isolates demonstrated that they present divergent features. This study was undertaken in order to clarify the phylogenetic relationships among O113:H21 E. coli strains from different sources, circulating in Brazil.

Material and Methods: Thirteen O113:H21 isolates, previously evaluated by Pulsed-Field Gel Electrophoresis (PFGE) and presenting distinct Xhol restriction patterns were analyzed by multi locus sequence typing (MLST). For this purpose the EcMLST scheme (www.shigatox.net/mlst) based on the sequencing of the seven housekeeping genes (aspC, clpX, fadD, icdA, lysP, mdh, and uidA) was used. Toxinotyping (stx1, stx2, stx2-negative) was performed by a method detecting VT of vtx genes. For this purpose the EcMLST scheme (www.shigatox.net/mlst) based on the sequencing of the seven housekeeping genes (aspC, clpX, fadD, icdA, lysP, mdh, and uidA) was used.

Results: Seven distinct MLST profiles were identified, and five of them represent novel sequence types (ST). Sequence types 223 and 846, which have already been assigned in the literature, and are related to highly virulent clones belonging to STEC clonal group (CG) 2 were found to be predominant in the majority of the studied strains. The phylogenetic tree based on seven housekeeping genes using neighbor-joining algorithm showed the occurrence of seven distinct clusters. One stx-negative human isolate clustered together with STEC strains belonging to ST 223 and CG 30.

Conclusions: The results obtained indicate that O113:H21 strains in Brazil are relatively diverse in relation to their phylogeny. However, human stx-negative O113:H21 isolates may have evolved from the same ancestral of the O113:H21 STEC.

P-041
Distribution of Genetic Markers Useful for the Identification of Shiga Toxin-Negative O26 Escherichia coli Strains Isolated in Brazil

Introduction and Objectives: Diagnosis of enteropathogenic (EPEC)/enterohemorrhagic (EHEC) E. coli infections is essential to prevent possible outbreaks and determine the best method of treatment. One of the virulence factors involved in their pathogenesis is the E. coli-secreted protein B (EspB), which can be a target for immunological detection. In the present study we established the ideal conditions for EspB expression and secretion in order to improve its detection by immunological assays.

Material and Methods: EspB expression was evaluated in 72 atypical EPEC, 35 typical EPEC and 25 EHEC isolates after growing in Dulbecco’s Modified Eagle Medium (DMEM) with 1% tryptone at different pH, in presence or absence of CO₂, at different incubation periods. Also, the effect of 0.1 M EDTA or 2% Triton-X100 addition on EspB secretion was studied. The culture supernatants were analyzed by ELISA using IgG-enriched fraction of rabbit anti-EspB polyclonal antibodies.

Results: The best condition for EspB expression was after growth in DMEM with 1% tryptone at pH 7.2, with 5% of CO₂ for 24 h. The
secretion of EspB was improved after incubation with 0.1 M EDTA for 1 h under constant shaking (250 rpm) allowing the EspB detection in 91% of typical EPEC, 83% of atypical EPEC and in 68% of EHEC strains. The expression of EspB by EPEC/EHEC strains and non-LEE bacterial isolates was statistically significant (Student t test, \( P < 0.05 \)) (Fig. 1).

Conclusions: In our results we determine the best condition for EspB expression and secretion, also demonstrating the use of our anti-EspB as a promising tool for detection of EPEC and EHEC strains.

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P-043
Development of an Immunochromatographic Test for Diagnosis of Shiga Toxin-Producing Escherichia coli (STEC)
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Introduction and Objectives: Detection of Shiga toxin-producing Escherichia coli (STEC) pathogen is extremely important not only to allow a correct treatment but also to prevent potential outbreaks in the community. Commercially available immunological test kits for STEC identification have been offered by different companies although some of them have shown variability in sensitivity and specificity when tested in different reference laboratories. The Vero cell toxicity test is often used as the gold standard test since it was established to establish the positive reaction and the optimal detection time. When all these parameters were set, MAb-collodial gold probe specific to the toxin was applied to glass fiber and anti-Stx2 polyclonal antibodies applied to nitrocellulose.

Results: The IC test strips were assembled using a sequence of three types of papers: cellulose fiber, glass fiber and nitrocellulose. After assembly, the strips were tested by immersion of the cellulose pad in its respective toxin. The toxin presented in the sample migrated and was captured by the polyclonal antibodies generating a red line. Reaction was considered positive when the sample and control test presented the characteristic red color. The IC test showed a positive reaction after 15 min and the detection limit was estimated as 60 ng/mL to Stx2.

Conclusions: The results demonstrated that antibodies employed could be used in an IC method. In addition, the developed method represents a promising tool for the rapid diagnosis and in the future could be applied for the detection of the involved pathogen.

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P-044
Characterization of Neutralizing anti-Stx1 and anti-Stx2 Monoclonal Antibodies: Distinctiveness Tools for Diagnosis and Therapy
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Introduction and Objectives: Monoclonal antibodies (MAbs) can be used for diagnosis and therapy of Shiga toxin-producing Escherichia coli (STEC) infections. However, numerous assays developed for STEC diagnosis showed variability in sensitivity and specificity when evaluated by reference laboratories and any therapy or vaccine are currently approved. Passive immunization strategies using humanized MAbs against STEC, which neutralize one or more Stxs toxins, are under investigation by several research groups. Thus, the aim of this work was the characterization of in-house produced MAbs against Stx1 and Stx2, in relation to their binding and neutralization abilities to Shiga toxins.

Material and Methods: Hybridomas were obtained after fusion of popliteal lymphnode cells from immunized mouse with detoxified Stx1 or Stx2 and SP2/O-Ag14 mouse myeloma cells. MAbs isotypization, detection limit, affinity constant and heat stability were characterized by ELISA and identification of the recognized subunit was detected by immunoblotting after MAbs subcloning. Moreover, MAbs binding ability to the toxin was also assessed by immunofluorescence after Stx1 or Stx2 Vero cell interaction. The neutralization test was performed using different MAbs concentrations against 50% of Vero cytotoxicity, which was determined as 1 and 50 ng for Stx1 and Stx2, respectively. Toxins cytotoxicity and neutralization assays were determined spectrophotometrically (\( \lambda = 595 \) nm) after 72 h of incubation and cells staining by crystal violet.

Results: Anti-Stx1 and Stx2 MAbs were classified as IgG1 and recognized only the A subunit of both toxins. The detection limit and immunoactivity of MAbs are described in Table 1. Also the MAbs binding ability was visualized after the toxins-Vero cell interaction by immunofluorescence. Besides 90% of neutralizing ability of Stx1 was achieved by anti-Stx1 MAb up to...
Typing of Verocytotoxin-Producing Proficiency Testing for the Detection and Development of Methods and Organization of (VTEC) toxins.

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100 ng; on the other hand, 63% to 53% of Stx2 inhibition required 50 and 20 μg of anti-Stx2 MAb, respectively. The cellular integrity was maintained on these antibodies concentrations in absence of toxins.

Table 1 Dissociation constant, detection limit and immunoreactivity of anti-Stx1 and anti-Stx2 MABs

| MABs characteristics | Stx1 | Stx2 |
|----------------------|------|------|
| Dissociation constant (Kd) | 3.4 × 10⁻¹⁰ M | 6.14 × 10⁻¹⁰ M |
| Detection limit (200 μg) | 6.2 ng | 12.5 ng |
| Stability (temperature) | 50°C | 70°C |
| Total loss of immunoreactivity (80°C) | 1 min | 5 min |
| Partial loss of immunoreactivity | 60 and 70°C | 80 and 90°C |

Conclusions: In conclusion, the immunochemical characteristics of the MABs analyzed point at their use in STEC diagnosis. Also, their neutralizing abilities against the Shiga toxins make these MABs promising tools for STEC therapy.

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P-045
The European Union (EU) Reference Laboratory for Escherichia coli: 5 Years Experience on the Development of Methods and Organization of Proficiency Testing for the Detection and Typing of Verocytotoxin-Producing E. coli (VTEC)

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Introduction and Objectives: The EU-RL for VTEC was established in 2006 by the EC Directorate General for Health and Consumers (DG Sanco), according to the Regulation (EC) No. 882/2004 on official controls. It coordinates a network of 32 EU National Reference Laboratories (NRLs) and the main objective of its mandate is to ensure that the methods for the identification and typing of E. coli strains as VTEC used by the NRLs are standardised, as well as the methods for the detection of these pathogens in food and animal samples.

Material and Methods: The EU-RL accomplishes its mandate by developing and evaluating methods, distributing reference materials, organizing proficiency tests, and hosting scientists from NRLs for training stages. The EU-RL VTEC also collaborates with other EU structures (EFSA, ECDC) in establishing monitoring and surveillance programs for VTEC.

Results: Since 2006, the EU-RL has developed and evaluated standard operating procedures for the identification and typing of VTEC and for their detection in food mainly based on PCR detection of virulence genes. In particular, it coordinated the development of a CEN ISO Technical Specification on the detection of VTEC in food and animal feed, based on the Real Time PCR screening of food enrichment cultures and aimed at the detection of the VTEC serogroups most associated with severe human infections. To evaluate both the methods and the performance of the NRL network in their application, the EU-RL organized seven rounds of proficiency testing (PT). Four PT were dedicated to bacterial typing and involved the detection of VTEC virulence genes by PCR and the identification of the serogroups most involved in human disease in Europe both by serological and molecular methods. Four PTs were dedicated to the detection of VTEC in different matrices, including carcass swabs, milk, and vegetables, by using the Real Time PCR-based ISO-CEN Technical Specification. A positive trend was observed in both the number of participating laboratories and their performance.

Conclusions: The control of pathogenic VTEC in food and animals represents a challenge for the development of specific detection methods and requires a network of skilled and trained laboratories throughout the EU for their detection in the vehicles of infection. The EU-RL is working to consolidate such a network, in order: (i) to contribute to the knowledge of the epidemiology of VTEC infections in Europe; (ii) to gather harmonized data on the prevalence of these pathogens in the food samples finalized to the definition of microbiological criteria for VTEC; (iii) to provide the EC with more standardized operative structures and tools to face possible emergencies in this field of food safety.

P-046
Surveillance of Non-O157 STEC Isolates in the Netherlands 2007–2011

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Introduction and Objectives: In the Netherlands, an intensified surveillance of STEC O157 has existed since 1999. In 2007, the scope of this surveillance was widened to include non-O157 STEC isolates.

Material and Methods: For this purpose, a real time PCR method was developed in 2006 to test for the presence of shigatoxin genes directly in faecal samples. From 2007, laboratories were encouraged to use this method and submit up to five cultured isolates (sorbitol positive or negative) from a PCR positive faecal sample to the RIVM in an effort to obtain the isolate responsible for the positive signal. These isolates were tested for the presence of shigatoxin and other virulence genes and when found positive, they were further typed with serotyping. Over the years, more laboratories included a PCR in their routine diagnostic workup.

Results: During 4 years (2007–2010) of STEC surveillance, this strategy resulted in submission of 3536 isolates from 703 different patients. Only for 193 patients (27.5%), an isolate could be found harbouring either the shigatoxin 1 or 2 gene. When isolates harbouring virulence genes eae or hly are also included, this success rate climbs to 32.6% (229 patients). The preliminary results from 2011 indicate a significantly higher success rate (47.5%) for finding a shigatoxin gene positive isolate. In only 12 of these patients (6.2%), the isolate found was O157, but O157 is also submitted based on sorbitol negativity on SMAC, so this does not reflect the prevalence of O157 in the general population. Most frequently isolated serotypes are O63 (11.4%), O26 (10.9%) and O91 (7.3%). Remarkably, shigatoxin subtype 2f, which was added as a target for PCR in 2008, was found in 21.2% of all positive isolates. This subtype was first found in pigeons and is not (yet) widely regarded as a major pathogen. From four patients, an STEC isolate was obtained more than once, with 2 weeks to 6 months time between sampling dates.

Conclusions: The increased success rate of isolating an STEC isolate in 2011 is most likely caused by a change in submission criteria. Because the success rate of isolation decreased as the Ct value found in the faecal samples increased, laboratories were requested not to submit random cultured isolates from samples with a high Ct value, i.e. >35 in the beginning of 2011. Even though the number of found O157 isolates using this strategy does not represent prevalence in the general population, it is clear that diagnostic methods should also include non-O157 STEC. However, the clinical relevance is not well-established for all non-O157 STEC serogroups. A new insight that this surveillance strategy has provided is the high occurrence of
serotype O63, which carry subtype stx2f, and other STEC harbouring stx2f in the Netherlands. This shiga toxin subtype has also been described to be emerging in Germany but only occasionally in other countries.

P-047
Rapid detection of Shiga Toxin 2-Producing Escherichia coli O104:H4 Outbreak Strain Using Real-Time Multiplex PCR Assay
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Introduction and Objectives: A large outbreak of hemolytic-uremic syndrome (HUS) and diarrhea caused by Shiga toxin (Stx) 2–producing Escherichia coli O104:H4 occurred in Germany in May to July, 2011. Timely identification of new cases required rapid detection of the outbreak strain. We developed a real-time multiplex PCR (real-time MPCR) which targets typical molecular features of the outbreak strain and determined its utility for the identification of the outbreak strain.

Material and Methods: The real-time MPCR primers target stx2, and wzyO104 and fliC144 genes which are parts of the O104 and H4 antigen clusters. The assay was performed using Fast EvaGreen SuperMix in a CFX96™ Real-Time PCR Detection System. The amplifications were determined by melting point curve analysis. A total of 234 enterohemorrhagic E. coli (EHEC) strains (91 O104:H4 outbreak isolates, 42 strains of the HUSEC collection, and 101 EHEC belonging to a broad spectrum of serotypes and stx subtypes) and 137 stool enrichment cultures from patients involved in the outbreak were analyzed.

Results: All EHEC O104:H4 strains (91 outbreak isolates and HUSEC041) were correctly identified in the assay. The melting point curve analysis demonstrated three peaks, at 75°C for wzyO104, 80.2°C for stx2 and 84°C for fliC144. Two other strains of the HUSEC collection which contain wzyO104 in combination with fliC144 and stx2 gave specific amplicons for wzyO104 and stx2, but not for fliC144. Among 101 other EHEC, only four strains which possess O104 or H4 antigen produced the wzyO104 or fliC144 amplicons, respectively. Eighty-three from 137 enrichment stool cultures contained the outbreak strain as detected by conventional PCR and all of them produced at least three amplicons in the real-time MPCR.

Conclusions: The real-time MPCR is a rapid, sensitive and specific method for the detection of EHEC O104:H4 in human stools and identification of the isolates.

P-048
Genetic Characterization of non-O157 Verocytotoxigenic Escherichia coli Isolated from Sheep Using Multiple-Locus Variable-Number Tandem Repeat Analysis (MLVA)
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Introduction and Objectives: Verocytotoxigenic Escherichia coli (VTEC) can produce serious human illness linked to the consumption of contaminated food, mainly of bovine origin but also of small ruminant origin in some countries. Serogroup O157 especially represents a major public health concern worldwide. However, multiple reports have shown that VTEC strains belonging to other serotypes (non-O157 VTEC) have been implicated in numerous outbreaks in some countries as a cause of severe diseases in a proportion similar to O157 VTEC strains. Recently, many studies have been performed about multiple-locus variable-number tandem repeat analysis (MLVA) in O157 VTEC strains, but there is a lack of information about MLVA in non-O157 VTEC strains. This work aimed to investigate the genetic diversity among epidemiologically related non-O157 VTEC isolates from sheep using MLVA.

Material and Methods: MLVA was used to genotype a total of 117 VTEC isolates from sheep deriving from four different farms without epidemiological relations in Spain during a period of one year. VTEC isolates belonged to three different serogroups: O5 (24 isolates), O146 (40 isolates) and O91 (53 isolates). These serogroups have been frequently identified among ovine VTEC strains and associated with human strains that have caused haemolytic-uremic syndrome. MLVA was performed as described by Lindstedt et al. (Journal of Microbiological Methods. 2007, 69: 197–205) with little modifications. Seven variable number of tandem repeats (VNTR) loci were amplified using this method (CVN001, CVN002, CVN003, CVN004, CVN007, CVN014, and CVN015) and PCR products were analyzed by capillary electrophoresis. The isolates had previously been analyzed by PCR for genes encoding verocytoxins 1 and 2 (vtx1 and vtx2), intimin (eae) and enterohaemolysin (ehxA).

Results: All the seven primer sets but one amplified all isolates at all loci and from one to 11 alleles were found for the six loci. The isolates could be principally discriminated by alleles of four of the seven studied (CVN001, CVN004, CVN007 and CVN0014), while CVN003 locus rendered null alleles in all the isolates. Several MLVA profiles were found in different serogroups. O91 serogroup was correctly differentiated in a different cluster from the other serogroups, while O146 and O5 were classified in the same cluster. Among the O91 VTEC isolates, 13 different profiles were observed. The O5 VTEC isolates showed seven different profiles and finally, among O146 VTEC isolates five different profiles were observed.

Conclusions: This study corroborates the suitability of the MLVA for genotyping of non-O157 VTEC belonging to serogroups O91, O146 and O5. It also suggests the need to increase the number of loci which could allow a higher discrimination among isolates and serogroups.

P-049
Colonie Immunoblotting and Hybridization for Isolation of all and Selected Serotypes of Verocytoxin-Producing Escherichia coli (VTEC)
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Introduction and Objectives: Non-O157 VTEC are a rising concern as foodborne/waterborne pathogens. While five or six ‘priority’ serogroups cause 70–80% of serious non-O157 VTEC infections, others also cause serious illness and many have little or no health risk. Hence, risk management of VTEC-contaminated foods and water requires prompt and reliable VTEC isolation and characterization. However isolation of non-O157 VTEC is technically demanding since only VT isolates distinguish most VTEC from generic E. coli. Of available methods, variations on colony immunoblotting (CIB) and colony hybridization (CH) have been most successful. Here we compared CIB and CH for VTEC isolation from food cultures in two formats, spread-plated membranes (SPMs) and hydrophobic
grid membrane filters (HGMFs). We also investigated HGMF-CIB for concurrent identification of ‘priority’ VTEC and for isolation of VTEC from untreated, heavily impacted surface water.

**Material and Methods:** To compare CIB and CH, dilutions of food cultures inoculated with marker or other VTEC were plated on round SPMs and filtered onto HGMFs. The membranes were placed on antibody (Ab)-coated VT capture membranes on agar plates, or directly on agar plates for 18 h incubation. In CH, VT genes in colony lifts from the SPMs or in colonies on replicated HGMFs were detected with DNA probes. In CIB, the SPMs and HGMFs were reserved and the underlying VT capture membranes were probed with VT Abs. Evaluation included rates of isolation, specificity for VTEC, numbers of colonies isolated, clarity of staining, test simplicity and test time. For concurrent detection of priority VTEC, a second capture membrane placed between the HGMF and VT capture membrane during incubation was probed with Abs to O26, O111 and O157 antigens (others to be included). For water testing, HGMF-CIB was applied to enrichment cultures of filters from >0.5 L of surface water and to smaller volumes of the same samples filtered directly onto HGMFs for CIB without enrichment.

**Results:** Overall, HGMFs were superior, detecting 3–15 more VTEC colonies than SPMs by either CIB or CH. Both methods and formats recovered the inoculated strains, except for 1 strain by CH. CIB was simpler and faster, yielding isolates 48 h earlier than CH. HGMFs, having 1600 discrete cells delineated by hydrophobic grid lines provided more sensitive and reliable isolation of low numbers of VTEC among numerous background bacteria. Concurrent detection of O26, O111 and O157 VTEC in mixed cultures of other VTEC and non-VTEC by HGMF-CIB was very successful. In 101 water samples, HGMF-CIB testing without filter enrichment yielded far more positives than after enrichment (32% versus <1%).

**Conclusions:** The HGMF-CIB method is a fast and reliable method for isolation of all and selected VTEC serotypes.

**P-052**

**Rapid Field Based Detection of STEC O104:H4 by Loop Mediated Isothermal Amplification (LAMP)**

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**Introduction and Objectives:** Shiga Toxin producing *Escherichia coli* O104 is a rare cause of foodborne illness in humans in the European Union (EU) and worldwide. The recent outbreak of *E. coli* O104:H4 in Germany and France during the summer of 2011 (which is recognized as the deadliest outbreak of STEC ever recorded) highlights the need for new and novel rapid diagnostics to detect this pathogen directly in the field, during food production. The objective of this work was to develop a rapid Loop Mediated Isothermal Amplification (LAMP) assay to effect rapid detection of STEC O104:H4 in the field.

**Material and Methods:** The STEC O104 outbreak strain was obtained from the American Type Culture Collection (ATCC). Other bacteria used in this study as negative controls included foodborne outbreak isolates of Enterohemorrhagic *E. coli* (EHEC) (*E. coli* O104:H7, *E. coli* O22:H8, *E. coli* O26:H11), *E. coli* JM109, and *Salmonella enterica* serovar Typhimurium. The isolates were screened with a newly developed multiplex LAMP assay for characteristic features of the outbreak strain (rboO104, flIC4H, stx2, aggR, and terD). Other STEC virulence genes (stx1, stx2, eae, and *E. coli* specific genes (dnaK, positive control) were also included in the LAMP assay. The first set of experiments evaluated the assay using purified genomic DNA from all bacterial isolates. For the field assay, 3 L of vegetable irrigation water were spiked with low levels of STEC O104:H4. The entire volume of water was concentrated by using a peristaltic pump to pass the water through a DIF-MN30 filter at a flow rate of 300 mL/min. Following filtration, the filter was removed from the filter cartridge followed by direct DNA isolation from the filter. The LAMP assay then proceeded.

**Results:** The results indicated that STEC O104:H4 genomic DNA could be detected within 10 min, with all STEC O104:H4 specific target (rboO104, flIC4H, stx2, aggR, and terD), being detectable within 10–12 min. The detection limits of the targets from genomic DNA ranged from as little as 1 pg (stx2 (within 50 min)) to 100 pg

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[rfbO104 (within 40 min)]. In contrast, the non STEC O104:H4 targets (stx1, eae) were not detected. The non STEC O104:H4 bacteria did not exhibit positive results for amplification of the STEC E. coli O104 specific targets, with the exception of the terD gene and the stx2 gene. EHEC isolates have been previously shown to carry both genes. When E. coli O104:H4 was concentrated from vegetable irrigation water followed by detection by the multiplex LAMP assay, all specific targets were detected within 15 min indicating that the entire assay could be completed from start to finish within 1 h.

**Conclusions:** This study demonstrates the utility of a rapid molecular based assay that can be used directly in the field to assess contaminated food production sites for emerging foodborne pathogens.

**P-053**  
**A Review of Faecal Testing for VTEC in Scotland**

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**Introduction and Objectives:** In Scotland, local diagnostic laboratories test faeces for E. coli O157 by direct culture, and forward negative samples from selected patients to the Scottish E. coli O157/VTEC Reference Laboratory (SERL) for additional more sensitive tests, in line with Scottish health protection guidance. We reviewed the added value of these SERL tests i.e. how many additional VTEC cases were microbiologically confirmed, and compared the clinical and cost effectiveness of the current SERL methodologies for detecting VTEC in faeces.

**Material and Methods:** Over an 18 month period, SERL received 4456 faecal samples. For each, an enrichment broth was tested by both IMS for E. coli O157, and PCR to detect vtx1, vtx2, eae, hly, rfbO157, sfp genes. We determined the number of cases identified solely as a result of faecal testing at SERL, and the number of cases identified by each method (IMS and PCR). Information on the severity of illness in each IMS positive/PCR negative case was obtained from the clinical details supplied to Health Protection Scotland.

**Results:** A total of 469 individuals testing positive for E. coli O157 or non-O157 VTEC were identified in Scotland over the 18 month period, with 111 (24%) VTEC infections detected solely by faecal testing at SERL. Fifty-four of these were positive for non-O157 VTEC. 56 for E. coli O157, one was positive for both E. coli O157 and O111, and one for both E. coli O26 and O145. PCR alone detected more positives than IMS, but 14/469 E. coli O157 infections would not have been microbiologically diagnosed if the faecal samples had only been tested by PCR. Of 14 cases identified solely by IMS, two were hospitalised (one with HUS) and in one case eae and hly genes, but not vtx1 or vtx2, were detected in the faecal sample. A number of these 14 were contacts of confirmed cases. Interestingly, following repeat testing of samples from the 14 original IMS-only positive cases which had been stored frozen at -80°C, only five tested positive for E. coli O157 by PCR but 11 tested positive by a loop-mediated isothermal amplification (LoopAmp) assay.

**Conclusions:** The number of faeces submitted to SERL continues to rise annually and the IMS-PCR combination, although effective, is time-consuming and costly. Results confirmed that IMS is more sensitive than the current SERL in-house PCR in detecting E. coli O157. However, testing by IMS alone would have reduced the number of microbiologically confirmed cases by 16%, whereas testing by PCR alone would have reduced the number of confirmed cases by only 3%. By improving the extraction efficiency of DNA from faeces and introducing real-time PCR, SERL intends to increase the speed and sensitivity of detection in addition to reducing unnecessary work and costs. Further evaluation of the LoopAmp assay is also under-way, as this may be a useful adjunct to current VTEC testing at diagnostic laboratories which do not have access to PCR testing.

**P-055**  
**Characterization of Shiga Toxin-Producing Escherichia coli Isolated from Urine Samples of Patients Suffering from Urinary Tract Infection**

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**Introduction and Objectives:** The vast majority of uncomplicated urinary tract infection (UTI), the most common type of bacterial infection in industrialized countries, is caused by uropathogenic Escherichia coli (UPEC), a subtype of extraintestinal pathogenic E. coli (ExPEC). Although many E. coli pathogens can be categorized into different pathotypes based on their virulence gene content, there is much overlap in the mechanisms underlying pathogenesis. Many UPEC isolates, especially isolates from nosocomial UTI, cannot be unambiguously distinguished from certain intestinal pathogenic E. coli (IPEC) or from commensals. We aimed at the characterization of atypical UPEC isolates from nosocomial urinary tract infections.

**Material and Methods:** We analyzed 102 E. coli isolates from hospital patients with urinary tract infection. These isolates were screened by multiplex PCR for important virulence determinants of UPEC and IPEC. Furthermore, these isolates were subjected to molecular epidemiological analysis by multi locus sequence typing (MLST) and selected strains were phenotypically compared with regard to biofilm formation, motility, adhesion to eukaryotic cells, and expression of virulence factors.

**Results:** Interestingly, three isolates carried virulence genes of Shiga toxin-expressing E. coli (STEC) and also expressed typical STEC virulence factors such as Shiga toxin and EHEC hemolysin. These strains differed in their STEC virulence gene repertoire, serotype, phylogenetic background and virulence- or fitness-associated phenotypes.

**Conclusions:** Our results indicate that nosocomial UTI due to E. coli may be caused by a diverse group of E. coli variants, some of which represent STEC. As UPEC strain typing and risk assessment does not include detection of STEC and other IPEC virulence markers, our findings may be important for improved and efficient diagnostic approaches. The potential of STEC to colonize and infect the urinary bladder and molecular mechanisms involved remain to be investigated.

**P-056**  
**Inhibition of Polymerase Chain Reaction can Prevent Detection of Escherichia coli O157:H7 in Walnuts**

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**Introduction and Objectives:** Shelled walnuts are a food that does not have a high association with E. coli O157:H7, but associated illness and contamination has recently been reported in Canada. Most methods for the routine analysis of foods for E. coli O157:H7 were originally developed for use with meat products. Consequently, there has been little evaluation of methods of analysis for its detection in walnuts. The aim of this study was to determine whether E. coli O157:H7 can be reliably detected and isolated from walnuts using standard methods.

**Material and Methods:** To determine whether interference with PCR detection occurs in, triplicate experiments, 65 g walnut samples
were inoculated with *E. coli* O157:H7 at low (3, 6 or 15 CFU), medium (30, 60, or 150 CFU) and high (300, 600, 1500 CFU) levels. Samples were suspended in 585 mL of modified tryptose soy broth with novobiocin 20 mg/L (mTSB-n) and incubated for 24 h at 42°C. *E. coli* O157:H7 in mTSB-n was prepared as a positive control. Negative controls were uninoculated mTSB-n and uninoculated walnuts. Following enrichment, the presence of *E. coli* O157:H7 was confirmed by isolation onto modified HC agar with tellurite and cefsulodine. Template for PCR analysis was prepared from enrichment broth by DuPont Qualicon BAX system. *E. coli* O157:H7 MP, BioRad InstaGene Matrix and Qiagen DNeasy Blood and Tissue Kit. The BAX prepared template was analyzed using the BAX system. The other two templates were analyzed by PCR for verotoxin genes. Additional template was prepared from the 3 CFU/65 g enrichment broth and serially diluted in 10 mM TE buffer.

**Results:** The enrichment broths of all inoculated walnut samples confirmed positive for *E. coli* O157:H7 by isolation. *E. coli* O157:H7 was not isolated from either of the uninoculated negative controls. BAX analysis detected *E. coli* O157:H7 in four walnut samples (150, 300, 600 and 1500 CFU), but gave false negative results for the other samples of inoculated walnuts. InstaGene Matrix template gave false negative results for all inoculated walnut samples. DNeasy Kit template gave false negative results, except for two samples (60 and 150 CFU). BAX template from the 3 CFU/65 g sample was diluted up to 1:100 000 did not test positive. Verotoxin genes were detected in three replicate tests of InstaGene Matrix template diluted 1:100 and 1:1000 and Dneasy Kit template diluted 1:10, 1:100 and 1:1000.

**Conclusions:** There is no evidence that walnut inhibits the isolation of *E. coli* O157 by standard culture methods. Inhibition of PCR reactions by templates from walnut enrichment was observed. Inhibition of PCR reactions from InstaGene Matrix and Dneasy Kit template can be overcome by dilution of template 1:100 in TE buffer prior to analysis. The use of PCR methods for the detection of pathogens in enrichment broths from walnuts is not recommended unless the template extraction method used has been optimized and validated.

### Development of Three Multiplex PCR Assays Targeting the Most Clinically Relevant Serogroups Associated with Enterohaemorrhagic *Escherichia coli* (EHEC)

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**Introduction and Objectives:** *Escherichia coli* serogroups O157, O21, O26, O45, O55, O91, O103, O111, O113, O118, O121, O123, O128, O145, O146, O157, O165, O172 and O177 are the most clinically relevant enterohaemorrhagic *E. coli* (EHEC) serogroups based on the severity of disease, frequency of reported illness and association with outbreaks. The aim of this work was to develop three multiplex PCR assays to detect those serogroups.

**Material and Methods:** Unique sequences for each serogroup were identified on the wzx or wzy genes, both located on the O-antigen gene cluster. Oligonucleotide primers specific for wzxO103, wzxO111, wzxO21, wzxO26, wzxO45, wzxO55, wzxO91, wzxO103, wzxO110, wzxO111, wzxO113, wzxO118, wzxO121, wzxO123, wzxO126, wzxO145, wzxO146, wzxO157, wzxO165, wzxO172 and wzxO177 were designed and combined in three multiplex 5′-nuclease PCR assays.

**Results:** The assays were validated by testing a collection of strains belonging to the most common O:H serotypes of pathogenic *E. coli*, including EHEC strains isolated from human patients. Each strain generated one serogroup-specific fragment ranging between 111 and 829 bp, and the different amplicons could be distinguished by conventional gel electrophoresis.

**Conclusions:** The combination of these three multiplex PCR assays enables the reliable detection of genes encoding the O antigen in *E. coli* isolates belonging to the most clinically relevant EHEC serotypes. Significance and Impact of the Study: Molecular serotyping is a faster, simpler and less expensive technique than traditional serotyping, especially for laboratories where PCR is already a routine tool. Additionally, PCR enables the detection of O antigens even when they cannot be expressed by the bacteria. Nevertheless, the method here proposed needs a broad validation for what a double-blind assay is being implemented in order to evaluate the sensibility and specificity of these multiplex PCR assays and to compare their results with those obtained by traditional serotyping. Furthermore, multiplex PCR assays targeting the flc alleles for the detection of flagellar antigens (H-antigens)

### Development and Evaluation of Three Scorpion Probe-Based Multiplex Real-Time PCR Assays for the Detection of Shiga Toxin-Producing *Escherichia coli* Serogroups O26, O45, O103, O111, O121, and O145

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**Introduction and Objectives:** In addition to continued testing for *E. coli* O157:H7, new regulations in the United States require industry to begin monitoring for six non-O157 Shiga toxin-producing *E. coli* (STEC) serogroups, referred to as the ‘top-six’ non-O157 STEC (O26, O45, O103, O111, O121, and O145), as these have been most frequently associated with outbreaks and cases of food-borne illnesses in the U.S. These top six serogroups were recently declared as adulterants in beef by the USDA Food Safety and Inspection Service (FSIS), and testing for these serogroups in beef will begin in March, 2012. The purpose of this study was to conduct evaluations of three separate multiplex Scorpion probe-based PCR assays in a tableted format for the detection of the top-six STEC serogroups.

**Material and Methods:** The three multiplex assay configurations were as follows: Assay 1 – O26, O111, O121, and internal positive control (IPC); Assay 2 – O45, O103, O145, IPC; Assay 3 – stx1/stx2, IPC. Studies evaluating the sensitivity of each of the real-time assays were conducted using titrations of cell lysates. Cell lysates were also used to conduct initial inclusivity and exclusivity studies for each assay configuration.

**Results:** Sensitivity of each assay with cell titers in tryptic soy broth for each of the various PCR targets was shown to be ≥1.0 x 10^2 CFU/mL. Each assay was shown to be 100% inclusive for the strains tested (n = 20–50 per assay). Moreover, no cross-reactivity with closely related strains in any of the strains was observed.

**Conclusions:** These results demonstrate the feasibility of deploying a panel of three novel real-time PCR assay configurations for the detection and monitoring of STEC O groups, as well as the virulence genes, stx1, stx2, and stx2 genes. The approach demonstrated could easily be expanded to include additional multiplex assays should regulations continue to expand into other O groups or virulence gene markers. Finally, the performance of the detection method based on use of the Scorpion probe-based multiplex PCR assays was compared to the FSIS Microbiology Laboratory Guidebook (MLG 5B.01) method for detection of the top-six STEC in ground beef.
P-059
Comparison of Three Different Detection Methods for Shiga Toxin-Producing E. coli
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Introduction and Objectives: Food-borne pathogens, such as Shiga toxin-producing Escherichia coli (STEC), are a worldwide public health concern. STEC infections may lead to bloody diarrhea and progress to severe complications such as hemolytic uremic syndrome in around 10% of the cases. E. coli O157:H7 is the most commonly known STEC; however, the non-O157 STEC strains are also important pathogens as demonstrated by the O104:H4 outbreak this year. Currently, the majority of microbiology laboratories in Canada only detect STEC O157 based on culture; therefore the non-O157 STEC cases are being under diagnosed. In 2009, the Centre for Disease Control and Prevention recommended new guidelines to test for all STEC by enzyme immunoassay (EIA) or nucleic acid testing in response to increasing outbreaks caused by non-O157 STEC. The objectives of this study are to compare the sensitivity, specificity, and cost-effectiveness of detecting Shiga toxin (stx) genes in STEC using loop-mediated isothermal amplification (LAMP), conventional, and real-time (RT) PCR.

Material and Methods: DNA was extracted from overnight cultures of bacteria and used as template for the different amplification assays. The LAMP assay was optimized using stx1 and stx2 primers from Hara-Kudo et al. (2007). Conventional PCR was performed using established protocols from the WHO Collaborating Centre for Reference and Research on Escherichia and Klebsiella (Denmark) and followed by agarose gel analysis. RT PCR was carried out on the ABI 7500 FAST system (Applied Biosystems, ON, Canada) with primers and protocol previously established at our laboratory. Both conventional and RT PCR were standardized to run for 40 cycles. Sensitivity of the three amplifications were determined using a clinical strain positive for both stx1 and stx2 genes. In the specificity panel, five Gram positive and 21 Gram negative bacteria isolates were included. In addition to determining the sensitivity and specificity of each assay, consumable cost/test and turn around time (TAT) of the assay was also evaluated.

Results: LAMP had a sensitivity of 1 CFU for stx1, and 1 CFU for stx2, a TAT of 3 h and a cost/test of $15. RT PCR detected 1 CFU for stx1 and 10 CFU for stx2 with a TAT of 65 min and a cost/test of $9.30. Conventional PCR detected 1 CFU of stx1 and 1 CFU of stx2 in 4 h and the cost/test was $13.50. Both RT and conventional PCR showed 100% specificity. LAMP was 100% specific, however; repeat testing was necessary for some organisms due to artifact formation.

Conclusions: All three methods had comparable sensitivities for stx1 and stx2. As LAMP requires only a heat block, it is much more cost effective as compared to the other assays which require expensive equipment. Therefore, LAMP had the best combination of sensitivity and cost-effectiveness.

P-060
Performance of Chromagar STEC for the Diagnosis of Diarrheagenic E. coli of Different Serotypes
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Introduction and Objectives: Detection of Shiga-Toxin producing E. coli (STEC) in the routine laboratory is challenging and has relied largely on screening for E. coli O157 in the past. The recent outbreak of an STEC O104 clone in Germany has underlined that STEC other than O157 are able to cause severe disease in humans including the hemolytic uremic syndrome (HUS). We examined a collection of STEC and other diarrheagenic E. coli (DEC) of different serotypes for growth on a new selective chromogenic medium in order to determine its value for routine laboratory STEC screening.

Material and Methods: Fifteen serotyped STEC strains and 32 E. coli belonging to other DEC pathogens were cultivated on Chromagar STEC (CS) (Mast Diagnostica, Reinfeld) and McConkey (MC) agar as by manufacturer’s instructions. Growth and colour of colonies were recorded after 24 and 48 h of incubation.

Results: Eleven of 15 STEC strains belonging to serogroups O26, O104, O145 and O157 grew on CS showing the typical mauve colour described by the manufacturer. Growth on CS was suppressed in four STEC strains (serogroups O91 and O103) although they were cultivable on MC. Six of 32 strains of other DEC pathogens also grew as mauve colonies (serogroups O55, O86, O126 and O127). All STEC strains of serogroups O26, O145 and O157 grew on CS whereas EPEC strains of the same serogroups did not.

Conclusions: The new Chromagar STEC is a useful selective medium for the most common serotypes of STEC. Few STEC (serogroups O91 and O103) were suppressed in growth and would have been missed. Several strains of different serogroups of other DEC pathogens were also able to grow on CS. This data indicates that CS is a helpful tool for STEC diagnosis in the routine laboratory. However, it cannot fully replace the current diagnostic procedures for STEC detection.

P-061
Development of Diagnostic Test-Systems for Identification of Shiga Toxin Producing E. coli
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Introduction and Objectives: Outbreaks of E. coli O104:H4 induced hemorrhagic colitis and cases of hemolytic uremic syndrome in Germany and other European countries in the summer of 2011 and the possibility of the same cases in Russia made it necessary to create adequate test-systems and algorithms for E. coli O104:H4 extraction and identification.

Materials and Methods: For initial E. coli O104:H4 identification in clinical samples and food products special enrichment media (sodium dodecyl sulfate broth, MacConkey medium), containing cetoxatime (25 µg/mL), nalidixic acid (4 µg/mL) were used. A latex test-system was created to identify E. coli O104:H4 colonies after cultivation on the enrichment media. The system is capable of fast identification of the pathogen. After that, biochemical properties, antibiotic and bacteriophage susceptibility and the presence of fkh160, fkh161, stx2 genes were studied in the isolated strains. The gene searching was performed with multiplex PCR, based on E. coli O104:H4 DNA nucleotide sequence. The PCR system was effective in diagnosing sporadic illnesses caused by Shiga toxin producing E. coli (STEC) in Russia.

Results: Our institute has organized state-scale manufacturing of the latex- and PCR-test systems. E. coli O104:H4 specific bacteriophages for identification of STEC. Based on the test systems and our diagnostic scheme, ‘Laboratory diagnostic of STEC-induced illnesses and STEC detection in foods’ guideline was published. The guideline is used in Russian diagnostic laboratories. Manufacturing of the test-systems and creation of the methodological framework made it possible for our institute to organize extension courses for 115 doctors from different Russian regions.
Poster Presentations
Genetics and Virulence Factors

P-065
Hypervirulent Escherichia coli O157:H7 Strains that Cause Hemolytic Uremic Syndrome in Neuquén, Argentina
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Introduction and Objectives: In the last decade, the incidence of HUS (20–30 cases/100 000 children under age 5) in Neuquén Province was higher than the national rate, and more than 80% of the cases were associated with E. coli O157:H7 infection. However, in the same period, the rate of STEC O157 detection in diarrhea was low (0.3%). This allows us to hypothesize that the strains circulating in this area have a high pathogenic potential to develop HUS, and the clinical evolution would be too fast to be detected at the first stage of diarrhea. The research intended to study the prevalence of E. coli O157 strains of the hypervirulent clade 8 and the presence of putative virulence determinants.

Material and Methods: A total of 70 strains isolated from HUS cases were cultured in LB broth under inducing (0.5 µl/l) and non-inducing conditions. For phage determination, a real time PCR with hairpin primers was used (Riordan et al., 2008; 46:2070), and the putative virulence determinants were detected by PCR (Kulasekara et al., Infect. Immun. 2009; 77:3713).

Results: The most prevalent stx-genotypes detected were stx2/stx2c(vh-a) (78.6%) and stx2 (15.7%). All strains harbored eae and ehxA genes. By XbaI-PFGE, 53 macrorestriction patterns were identified with at least 75.9% similarity. Twenty-four isolates were grouped in seven clusters, while 46 strains showed unique patterns. The bacterial growth curves in the absence of induction and growth/lysis curves were constructed. Each experiment was performed at least twice.

Conclusions: This study demonstrated a very high prevalence of STEC O157 strains of the hypervirulent clade 8, in Neuquén. This value (91.5%) is the highest reported so far. Also, the association with different putative determinants is higher than that described elsewhere. This preliminary data could explain the particular epidemiology of STEC O157 infections with a rapid progression to HUS, as observed in Neuquén, Argentina.

P-067
Phage and Verotoxin Levels Related to Cytotoxicity Titors in vt2-Positive Isolates
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Introduction and Objectives: Verotoxins, the main virulence factor of VTEC, are encoded in temperate phages, being their expression and release generally related to the lytic cycle of the phage. Since VTEC strains collected in our laboratory present differences in cytotoxicity titers for Vero cells, our objective was to evaluate the relationship between these differences with the level of production of phages and verotoxin.

Material and Methods: We performed the analysis with selected vt2-positive isolates that belonged to either the serotypes O157:H7 or O145:H-, or carried the emergent vt2g-variant. Their verotoxicity titers were determined in a previous study. The isolates were cultured in LB broth under inducing (0.5 µg/ml mitomycin C) and non-inducing conditions. For phage quantification, supernatants were collected 3 h post induction and for verotoxin detection (by Ridascreen EIA), after ON incubation. The cultures were monitored spectrophotometrically every hour for the first 5 h post induction and growth/lysis curves were constructed. Each experiment was performed at least twice.

Results: The bacterial growth curves in the absence of induction were similar for all VTEC isolates, however, the bacterial growth/lysis curves differed when cultures were exposed to mitomycin C. All the isolates, except for two which were vt2g-positive, clearly evidenced bacteriolyis under this condition. By the double-layer agar method using E. coli DH5a as host strain, phages were detected in supernatants of all O157:H7 and O145:H- isolates, but among the vt2g-positive isolates only in those that evidenced bacteriolyis under induction. Phage plaques hybridized to a vt2 probe, except those from one vt2g-positive isolate. Higher phage titers were detected when the cultures had been induced and, in general, phage levels were related to cytotoxic titers. All O157:H7 and O145:H- isolates and one vt2g-positive isolate (FB 62), gave strong positive results when verotoxin production was evaluated. The other three vt2g-positive isolates tested negative, even under induction. As no differences were detected neither between induced and non-induced cultures nor among different VT2-producing isolates, dilutions of the supernatants were tested. By using diluted supernatants, FB 62 strain could be identified as the the weakest verotoxin producer, under both uninduced and induced conditions.

Conclusions: Phage and verotoxin titers in culture supernatants were closely associated with the cytotoxicity levels of the isolates, showing an increase under induction. Curiously, one vt2g-positive isolate with low verotoxin titers rendered phage plaques that did not...
not correspond to vt2-phages and no verotoxin production could be detected by EIA.

**P-068**

**Integration Sites of Stx prophages in Human and Animal EHEC O157:H7 and O26:H11 Chromosome Could Reflect Their Virulence**

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**Introduction and Objectives:** Enterohemorrhagic *Escherichia coli* (EHEC) are zoonotic pathogens responsible of hemorrhagic colitis and the life-threatening hemolytic-uremic syndrome in humans. Infections are mainly acquired through consumption of contaminated foodstuffs and only a limited number of serogroups have been identified from severe diseases including O157 and O26 which are the most frequently reported worldwide. The cardinal virulence trait of EHEC is the production of Shiga toxins that are encoded by Stx prophages. Whole genome analysis of EHEC O157:H7 revealed a high degree of genetic diversity largely due to acquisition and lost of prophages. It is known that Stx prophages could insert in a limited number but preferential sites in the EHEC chromosome. Our study aims at comparing integration sites of Stx prophages of chromosome in O157 and O26 isolated from humans and animals to better understand the emergence of new pathogenic EHEC clones.

**Material and Methods:** A total of five human and 14 animal EHEC O157:H7 and O26:H11 strains were studied and compared by optical mapping analysis. This tool provides a graphical representation of the restriction sites location in the whole genome of these organisms and consequently allow to quickly and easily identify insertions, deletions, inversions and genomic rearrangements.

**Results:** Our results have demonstrated that *E. coli* O26:H11 show more diversity and complex chromosomal rearrangements than O157:H7 strains. Besides, several hotspots of rearrangements were detected in EHEC O157:H7 and O26:H11. Strikingly, the position of Stx prophage seems to be dependant both stx variants and of the number of Stx prophages integrated (Figure 1).

**Conclusions:** We could therefore hypothesize that these positions reflect the virulence potential of the strains and may be used as predictors of their pathogenicity.

**Figure 1:** Variation in the integrations sites of Stx prophages in the chromosome of human and animal strains.

**P-069**

**Variations in Shiga Toxin-Producing Abilities of Bovine *Escherichia coli* O157 Strains in Bangladesh**

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**Introduction and Objectives:** In Bangladesh, *E. coli* O157 is widely prevalent in animal reservoirs, but no human cases have yet been identified suggesting a possible low/no virulence potential of animal strains. It has been demonstrated that isolates possessing the stx2 gene that produce little or no toxins [toxin non-producing (TNP)] are widely distributed in various Asian countries. The objectives of the study were to assess the occurrence of TNP strains among *E. coli* O157 isolated from bovine sources in Bangladesh and to characterize the stx2 encoding bacteriophages induced from these strains.

**Material and Methods:** A total of 47 *E. coli* O157 strains that contained stx2 gene were tested for TNP genes by PCR and stx2 production was evaluated by reverse passive latex agglutination test (VTEC-RPLA). The presence of stx2 bacteriophages was analysed upon mitomycin C induction. Strains carrying stx2 phages were screened by plaque hybridization with stx2-probe. Phage DNA was extracted, characterized, and toxpin production was assessed after induction. Assays to obtain lysogens from different strains were also carried out and phages induced from the lysogens were compared with those induced from the *E. coli* O157 strains.

**Results:** All strains were positive for TNP genes, of which 87% (*n* = 41) showed either very low titers or no toxin. The remaining 13% strains (*n* = 6) showed moderate to high titers of the toxin. Inducible bacteriophages were detected only in the toxin-producing strains. PCR and Southern hybridization of phage DNA extracted from these strains confirmed that all contained the stx2 gene. Although these strains differed significantly in their induction level with mitomycin C, a linear relationship between phage induction and toxin production was observed in all strains. The size of phage genome ranged from 50 to 70 kb and EcoR1 digestion of the genome produced heterogeneous patterns, which are indicative of their genomic diversity. The stx2 gene was located in a single fragment of 8–10 kb in all phages. Phages varied in their capacity to infect different bacterial hosts. Stable lysogen was constructed in *E. coli* MC 1061 with one of the six phages (17%). The lysogenized strain was able to produce active Stx2 and had similar levels of phage induction as the wild-type strains.

**Conclusions:** Majority of *E. coli* O157 strains in Bangladesh are toxin non-producing (TNP). Despite the presence of TNP genes, a significant number of strains can produce high titers of Stx2, which is attributable to the presence of inducible stx2 phages. The diversity of stx2 phages and their capacity to infect and lysogenize in different bacterial hosts accentuate the risk of spreading stx and the emergence of new bacteria-producing Shiga toxin.

**P-071**

**Characterization of a Novel vtx2-Converting Phage Associated with Verocytotoxin-Producing *Escherichia coli* (VTEC) O157 Isolated from Human Infections**

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**Introduction and Objectives:** In Bangladesh, *E. coli* O157 is widely prevalent in animal reservoirs, but no human cases have yet been identified suggesting a possible low/no virulence potential of animal strains. It has been demonstrated that isolates possessing the stx2 gene that produce little or no toxins [toxin non-producing (TNP)] are widely distributed in various Asian countries. The objectives of the study were to assess the occurrence of TNP strains among *E. coli* O157 isolated from bovine sources in Bangladesh and to characterize the stx2 encoding bacteriophages induced from these strains.

**Material and Methods:** A total of 47 *E. coli* O157 strains that contained stx2 gene were tested for TNP genes by PCR and stx2 production was evaluated by reverse passive latex agglutination test (VTEC-RPLA). The presence of stx2 bacteriophages was analysed upon mitomycin C induction. Strains carrying stx2 phages were screened by plaque hybridization with stx2-probe. Phage DNA was extracted, characterized, and toxpin production was assessed after induction. Assays to obtain lysogens from different strains were also carried out and phages induced from the lysogens were compared with those induced from the *E. coli* O157 strains.

**Results:** All strains were positive for TNP genes, of which 87% (*n* = 41) showed either very low titers or no toxin. The remaining 13% strains (*n* = 6) showed moderate to high titers of the toxin. Inducible bacteriophages were detected only in the toxin-producing strains. PCR and Southern hybridization of phage DNA extracted from these strains confirmed that all contained the stx2 gene. Although these strains differed significantly in their induction level with mitomycin C, a linear relationship between phage induction and toxin production was observed in all strains. The size of phage genome ranged from 50 to 70 kb and EcoR1 digestion of the genome produced heterogeneous patterns, which are indicative of their genomic diversity. The stx2 gene was located in a single fragment of 8–10 kb in all phages. Phages varied in their capacity to infect different bacterial hosts. Stable lysogen was constructed in *E. coli* MC 1061 with one of the six phages (17%). The lysogenized strain was able to produce active Stx2 and had similar levels of phage induction as the wild-type strains.

**Conclusions:** Majority of *E. coli* O157 strains in Bangladesh are toxin non-producing (TNP). Despite the presence of TNP genes, a significant number of strains can produce high titers of Stx2, which is attributable to the presence of inducible stx2 phages. The diversity of stx2 phages and their capacity to infect and lysogenize in different bacterial hosts accentuate the risk of spreading stx and the emergence of new bacteria-producing Shiga toxin.
Introduction and Objectives: The existence of sub-populations of VTEC O157, which may be more virulent, has been proposed and the hypothesis is supported by the uneven distribution of the phage types (PT) among human infections and animal isolates. In Europe, PT21 is very common in strains isolated from cattle, however it is under-represented among human isolates, where PT8, PT2 and PT21/28 predominate. In the studies presented here we investigated the genetic differences of VTEC O157 strains from humans and animals belonging to PT8 and PT21.

Material and Methods: Ten VTEC O157 PT8 strains from humans and 10 PT21 strains from animals were compared by microarray. The polymorphic region of the VT2-phage was investigated by using a long PCR strategy followed by direct sequencing. The presence of the PT8-associated phage in 30 VTEC O157 PT21/28 strains of human and animal origin and in 78 strains belonging to different PTs was assessed using PCR primers targeting the region between the genes cro and ci.

Results: Microarray experiments showed that the main differences between the PT8 and PT21 strains were in the VT2-converting phage region. In particular, PT21 strains showed a phage structure almost identical to that of the VT2-phage from the reference strain EDL933, while all the PT8 isolates showed low or no hybridization in the segment identified by the gam-cII genes and encoding the machinery regulating the switch between lysis and lysogeny. A long PCR was used to amplify this region in order to investigate this polymorphism. For all the PT21 isolates a 6.1 kb amplicon was yielded whereas for all the PT8 strains a smaller 4.9 kb fragment was amplified. Cloning and sequencing of this region revealed that the VT2-phage present in PT8 strains was similar to the phage of the VTEC O157 strains implicated in the large Japanese outbreaks in the 1990s (Morioka and Sakai). We found that this particular phage was significantly associated with VTEC O157 isolated from human disease regardless of the PT. It was also present in most of the VTEC O157 strains belonging to PT21/28, which represents the most common PT isolated from disease in UK and has been associated with excretion at high levels from cattle.

Conclusions: We identified a VT2-converting phage in VTEC O157 belonging to PT8, which seems to be associated with VTEC O157 strains isolated from human cases of disease and with supershedding in cattle. Recent data show that the presence of VT-phages influences the expression of the type three-secretion system involved in the colonization of the host gastrointestinal tract. In the light of this observation, our findings suggest that the presence of this particular bacteriophage in VTEC O157 may influence the colonization of the gastrointestinal tract of ruminants determining a major exposure for humans.

P-073
Comparative Genomic Studies of Escherichia coli O157:H7 Strains Reveals Elements Associated with Up-Regulation of stx2 Production
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Introduction and Objectives: Production of Shiga toxin 2 (Stx2) by E. coli O157:H7 is a major contributor to the virulence of this pathogen. In previous studies, we have reported that human disease-associated lineage I and I/II O157:H7 strains have higher basal levels of Stx2 production than the primarily bovine-associated lineage II strains. Further, even within lineage I and I/II, human isolates were found to produce significantly more Stx2 than bovine isolates. In this study, we described a lineage I O157:H7 strain LRH6 which was associated with a laboratory-acquired infection and produces significantly higher levels of Stx2 than other members of this genotype.

Material and Methods: The DNA sequences of O157:H7 strains and their Stx2 prophage were either obtained from Genbank or by de novo 454 whole-genome pyrosequencing. Gap closure between contigs containing the Stx2 phage sequences was accomplished using long template PCR and subsequent sequencing of the amplicons. Stx2 protein expression was measured using ELISA and mRNA production by RT-PCR.

Results: LRH6 produced significantly higher amounts of Stx2 (7200 ng/mL) in overnight BHI cultures grown at 37°C than other O157:H7 strains in our collection for example, the outbreak-associated strains EDL933 and Sakai only produced 463 and 310 ng/mL, respectively. Similarly, stx2 mRNA expression by LRH6 was also significantly greater than that by EDL933 and Sakai (9.5 × 10^5, 6.6 × 10^3, and 2.9 × 10^5 copies/µL, respectively). The genome sequence of LRH6 had 99% nucleotide sequence identity with the Sakai genome, however, a two kb prophage-associated element encoding putative anti-repressor (ant) and TonB-like proteins was present in LRH6 but not the Sakai. Deletion of this element resulted in a seven fold reduction of Stx2 production by the mutant compared with the wild type LRH6 strain. Further, complementation of two EHEC virulence factors, namely the hemolysin from EHEC (EHEC-Hly) and the serine protease autotransporter EspPz. EHEC-Hly belongs to the family of RTX (repeat-in-toxin) and is a pore-forming cytolsin. It has been shown that EHEC-Hly lysed microvascular endothelial cells as well as erythrocytes, suggesting a role in pathogenicity. EspPz is believed to interfere with the blood coagulation cascade of the host by cleaving factor V and with the complement system by degrading and inactivating C3 and C5.

Results: Here, we demonstrate that EspPz cleaves EHEC-Hly and that this degradation abolishes the cytolytic activity on erythrocytes and microvascular endothelial cells in a cellular infection model. Functional inactivation of EHEC-Hly occurs when EspPz was supplemented to cultures expressing EHEC-Hly and when both virulence factors were coexpressed. EspPz is also able to degrade the more stable, vesicle-associated, form of EHEC-Hly, which was described recently by our group. Degradation of EHEC-Hly by EspPz occurs in the hydrophobic domain, which is essential for the interaction of the hemolysin with the host cell. EHEC in contact with human intestinal epithelial cells simultaneously upregulated EspPz and EHEC-Hly indicating that both molecules might interact under physiological conditions.

Conclusions: Our data suggest that interference of effector molecules might be an additional way to regulate virulence functions and bacterial pathogenicity.
the mutant with the ant gene on a multi-copy plasmid led to a two-fold increase in Stx2 production by the LRH6 mutant and Sakai. The tonB and ant elements were very common in lineage I O157:H7 strains and absent in lineage I/II and II strains. The Stx2 phage nucleotide and predicted amino acid sequence of Sakai and LRH6 were also identical except for three single nucleotide polymorphisms (SNP), one of which was non-synonymous and would be expected to cause an amino acid alternation in the phage CI repressor. Two E. coli K-12 strain LE392 lysogens infected with Stx2phage_Sakai and Stx2phae_LRH6 produced very low and similar amount of Stx2, suggesting that specific elements in the O157:H7 genome play an important role in Stx2 production.

**Conclusions:** While differences in the genomes of the Stx2-encoding phage in O157:H7 strains may affect the level of Stx2 production, regulatory factors located elsewhere in the bacterial genome also appear to play an important role in differential levels of expression of this important virulence factor.

**P-074**

**Plasmid Virulence Profiles of Verotoxigenic *Escherichia coli* Isolated in Argentina**

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**Introduction and Objectives:** The verotoxigenic *Escherichia coli* (VTEC) group is very diverse, being *E. coli* O157:H7 the most common serotype associated with sporadic cases and large outbreaks of hemolytic uremic syndrome in many countries. However, there is growing concern about the risk to human health associated with non-O157 VTEC serotypes. All clinical isolates of *E. coli* O157:H7 possess a large putative virulence plasmid called pO157 and similar plasmids are commonly found in non-O157 VTEC strains, presenting a considerable variability.

**Material and Methods:** We have characterized a total of 208 VTEC isolates from Argentina belonging to 49 serotypes, collected from cattle, foods and humans, for the presence of plasmid-encoded genes: epeA (serine-protease), espP (extracellular serine protease), katP (periplasmic catalase-peroxidase), stcE (zinc metalloprotease) and subA (subtilase cytotoxin). The epeA gene was detected by a monoplex PCR and the remaining ones, by a pentavalent PCR assay that also detects ehxA. The isolates had been analyzed in relation to other plasmid-encoded genes, ehxA and saa, in previous studies.

**Results:** The most commonly detected gene was espP, which was present in 182 (87%) of the VTEC strains tested. The gen katP was found in 36 strains (17%) whereas epeA, subA and stcE were present in frequencies ranging from 7% to 12%. The ehxA gene, present in 47% of the isolates, was usually detected in combination with other plasmidic genes. Those strains that were positive for subA harbored neither stcE nor katP; stcE was present only in O157:H7 strains, and katP never appeared simultaneously with saa. Taking into account the seven encoded-plasmid genes, 12 virulence profiles were also identical except for three single nucleotide polymorphisms (SNP), one of which was non-synonymous and would be expected to cause an amino acid alternation in the phage CI repressor. Two E. coli K-12 strain LE392 lysogens infected with Stx2phae_Sakai and Stx2phae_LRH6 produced very low and similar amount of Stx2, suggesting that specific elements in the O157:H7 genome play an important role in Stx2 production.

**Conclusions:** While differences in the genomes of the Stx2-encoding phage in O157:H7 strains may affect the level of Stx2 production, regulatory factors located elsewhere in the bacterial genome also appear to play an important role in differential levels of expression of this important virulence factor.

**P-075**

**Distribution of Serine Protease espP Subtypes Among Verotoxigenic *Escherichia coli* Isolated in Argentina**

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**Introduction and Objectives:** Infections by verotoxigenic *Escherichia coli* (VTEC) range from mild, self-limiting diarrhea to severe diseases such as hemolytic colitis (HC) and hemolytic uremic syndrome (HUS). Ruminants and derived foods are the main sources of human infections. Epidemiologic studies have shown that virulence factors other than verotoxins may also contribute to its pathogenicity in humans, including the plasmid-encoded serine protease EspP. EspP belongs to the serine protease autotransporters of Enterobacteriaceae (SPATE) family, and contributes to the adherence to bovine primary rectal cells and colonization of the bovine intestines. Moreover, degradation of human coagulation factor V via EspP could contribute to the mucosal hemorrhage observed in HC patients. Five espP alleles with different proteolytic activities have been identified and some authors have demonstrated that subtype α is associated with highly pathogenic serogroups.

**Material and Methods:** Since espP has been found in a high prevalence and there are few data about distribution of espP subtypes among VTEC strains from different origins, our aim was to investigate the occurrence of the subtypes α, β, γ, δ, and ε in espP-positive Argentine strains. Determination of espP alleles was done by PCR in 182 isolates belonging to 44 serotypes from cattle, food and humans.

**Results:** Some serotypes harbored only one espP subtype whereas others presented more than one allele. The α subtype was found in serotypes O26:H11, O38:H39, O103:NM, O103:H2, O115:H16, O145:NM, O146:NM, O157:H7 and O177:NM, whereas β subtype in O8:H16, O20:H19, O39:H49, O79:H19, O113:NM, O113:H21, O117:H7, O141:H8, O174:H21, O178:H19, O185:H7 and several ONT strains with different H antigens.

**Conclusions:** Subtyping of the espP gene revealed an association among O serogroups and particular espP subtype(s). Also, despite that in previous studies espP was present particularly in isolates from HUS patients, we found that some VTEC strains isolated from reservoir animals or foods also contained this espP allele. Particularly interesting is the observation that serogroup O103, considered to be highly pathogenic and reported in previous studies as negative for espP, resulted espP+ and carried the allele α.

**P-078**

**Comparative Genomics of Stx2 Prophages in O157:H7**

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**Introduction and Objectives:** The key virulence factors of enterohemorrhagic *Escherichia coli* (EHEC) are Shiga toxin types 1 and 2 (Stx1 and Stx2), each encoded by lambdoid prophages. EHEC O157 strains produce Stx1, Stx2, and Stx2 variant, Stx2c, alone or in com-
bimations. Stx2c has been further categorized into at least two subtypes, Stx2vha and Stx2vhb by such as PCR-restriction fragment length polymorphism (RFLP). It is thought that production of Stx2 is regarded as one of the risk factors for the severity of O157 infection because severe disease has been epidemiologically linked to the presence of Stx2 and purified Stx2 is 1000 times more toxic than Stx1 to human renal endothelial cells. The Stx2 gene is located downstream of the phage late gene promoter and its expression deeply depends on the phage regulatory system. Production and release of Stx2 primarily occur in a small fraction of the cell population in which Stx2 phage is spontaneously induced. Upon phage induction by mitomycin C (MMC), production and release of Stx2 are dramatically enhanced. While the level of Stx2 production is highly divergent among O157 strains either in the absence or presence of induction agent. Genomic diversity has also been found among Stx2 phages in O157 strains.

Results: In this study, we analyzed a total of 125 Stx2-positive EHEC O157 strains clinically isolated in Japan. By the RFLP analysis of stx2 gene, 49, 59 and 2 strains were found to contain stx2, stx2vha and stx2vhb, respectively. The remaining 15 strains were found to contain both stx2 and stx2vha and were excluded from further analyses. Of the 49 Stx2 phages, 39 were integrated in wrbA and 10 were in argW. All the 59 Stx2vha phages and all the 2 Stx2vhb phages were integrated in sbcB and yehV, respectively. We determined Stx2 production level of these strains in the presence or absence of MMC by using the VTEC-Reversed Passive Latex Agglutination (VTEC-RPLA) assay kit (Denka Seiken Co., Ltd., Tokyo, Japan). Although Stx2 was found to be produced by all strains, the level of Stx2 production was highly variable among the strains, even among the strains in which Stx2 phages are integrated in the same site. We classified strains into three (high, middle and low) or two (high and low) categories according to the Stx2 production level in each Stx2 phage integration site. Then, at least one representative strains of each category in each integration site, three integrated in wrbA, three in argW, four in sbcB and two in yehV, were selected and Stx2 prophages of these strains were sequenced. We also performed real time PCR analyses to determine stx2 mRNA level and copy number of stx2 gene in these strains. In this presentation, the results of genomic comparison in relation to the levels of phage induction, stx2 expression and Stx2 production of these O157 strains will be shown.

P-079
Identification of the Antiterminator q0111:H-gene in Norwegian Sorbitol-Fermenting Escherichia coli O157:NM K. Haugum1, K. Haugum2, B. A. Lindstedt2, I. Lobersil2, G. Kapperud2 and L. T. Brandal2
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Introduction and Objectives: Sorbitol-fermenting Escherichia coli O157:NM (SF O157) is an emerging pathogen suggested to be more virulent than non-sorbitol-fermenting Escherichia coli O157:H7 (NSF O157). The stx genes, encoding the Shiga toxins, are key virulence factors in both SF O157 and NSF O157 pathogenesis. Previous studies have suggested a correlation between the level of stx2 expression and the pathogenic potential of NSF O157. Antiterminator activity of the Q protein is important for the expression of stx2. Different q genes, qO111 and q21, have been identified in NSF O157, giving evidence of higher production of Stx2 in strains positive for the qO111 gene. Additionally, NSF O157 lineage I strains were shown to produce significantly more Stx2 than lineage II strains. Specific primers, the stx4 primer set, were found to differentiate these two lineages.

Concerning SF O157, the knowledge of stx2 expression and its regulation is sparse. We thus wanted to examine factors at the genomic level that might influence the expression of stx2 in SF O157.

Material and Methods: Seventeen stx2 positive Norwegian SF O157 were included in the study. The strains were stx2 subtyped and examined with different multiplex PCRs detecting, among others, the qO111 and q21 genes as well as the stx4 primer set. In addition the stx2 promoter region of all 17 isolates was sequenced. Three SF O157 were selected for further analyses and the sequences upstream (including the q gene region) and approximately 500 base pairs downstream of the stx2 gene were determined.

Results: The stx2EDL933 subtype was identified in all 17 SF O157. However, none carried the q21 or the qO111 genes, and all harboured mutations in the stx2EDL933 promoter region not seen in the NSF O157 strain EDL933 (GenBank AE005174). Additionally the majority of the SF O157 were negative for the stx4 primer set. Sequencing of the three selected SF O157 strains revealed that the antiterminator q gene and genes upstream of stx2EDL933 were identical or similar to the ones observed in the O111:H- strain AP010960, and different from the ones observed in the NSF O157 strain EDL933 (AE005174). Thus, distinct stx2EDL933 encoding bacteriophages between SF O157 and NSF O157 were suggested. Different DNA structures were observed among the three SF O157 strains sequenced, suggesting divergent stx2EDL933 encoding bacteriophages also within the SF O157 group. An assay for detecting qO111:H- was developed and all 17 SF O157 isolates were shown to carry this particular q gene.

Conclusions: In conclusion, the anti-terminator qO111:H- gene was identified in all our SF O157 and different stx2EDL933 encoding bacteriophages were suggested between SF O157 and NSF O157. However, further investigations are needed to elucidate whether the qO111:H- gene observed in our SF O157 contributes to high stx2 EDL933 expression, and thereby the increased virulence seen in SF O157 compared to NSF O157.

P-080
Identification of Virulence Genes and Correlation of Disease Outcome in Patients Infected with Shiga Toxin-Producing E. coli in Edmonton, Alberta, Canada
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Introduction and Objectives: Enterohaemorrhagic E. coli (EHEC) including Shiga toxin-producing E. coli (STEC) have been associated with food-borne outbreaks in the developed world causing bloody diarrhea, and hemolytic uremic syndrome (HUS) in 10% of the cases. Gene profiling of virulence factors provide molecular insights on their contributions to disease. The aim of this study was to establish genetic profiles of a subset of STEC O157 and non-O157 isolates in correlation with clinical outcome.

Material and Methods: A total of 113 isolates were included in the study; 89 were O157 STEC (chosen based on pulsed-field gel electrophoresis patterns from outbreaks and sporadic cases); 24 isolates were non-O157 STEC. Subtyping of the Shiga toxin gene (stx 1 and 2) was performed using established protocols from the WHO Collaborating Centre for Reference and Research on Escherichia and...
Klebsiella (Denmark). A set of genetic markers, including type III effectors, were tested by high-throughput microfluidic real-time PCR.

**Results:** There were 27 HUS cases (12 males and 15 females) with an age range of 19 months to 88 years, and median and mean ages of 2 and 8.9 years, respectively. Seven (26%) had culture positive stools for a minimum of 15 days and up to 62 days. Of the 89 O157 STEC isolates, 67 (75.3%) were positive for both stx1 and stx2, 19 (21.3%) positive only for stx2, and 3 (3.4%) were positive for stx1 and stx2. Five of them had HUS. The majority of isolates were positive for the virulence genes tested which included genes from the LEE, the O-island 71, O-island 122 and diverse type III effectors. However, three isolates were espP negative and one was nleF and nleA negative. The 24 non-O157 STEC isolates comprised of: 18 (75%) positive for stx1 -16 were stx1a and 2 were non typeable (NT) based on the primers used; 2 (8.3%) for stx1a and stx2; 2 (8.3%) for stx2; 1 (4.2%) for stx1 NT and stx2a; and 1 (4.2%) for stx2a. Only one person required hospitalization for rehydration and analgesia. Patient age in this group ranged from 6 months to 83 years. The most prevalent serotypes were O26:H11/HNM (17%) and O121:H19 (17%) followed by O111:HNM/NT (13%), O103:H25 (13%), O145:HNM (8%) and O5:HNM (8%). The virulence gene profiles vary within and between the serotypes.

**Conclusions:** The majority of the O157 STEC isolates were positive for all the virulence markers tested in this study in contrast to the non-O157 STEC. No difference was observed between the genetic profiles of isolates in the HUS and the non HUS cases. In the non-O157 STEC group, the profiles of the different serotypes varied but no conclusion can be drawn based on the limited number of cases studied.

**P-082**

**Cloning and Sequencing Cytolethal Distending Toxin V (cdt-V) Operon and Its Flanking Regions in an Atypical Bovine Escherichia coli O157:H43 Strain**

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**Introduction and Objectives:** Cytolethal distending toxins (CDT) are the prototype of inhibitory cyclomodulins. CDT is considered a relatively new virulence factor detected in several pathogenic bacteria. So far five types of CDT were recognized in *Escherichia coli* (CDT-I to V). CDT-V is the most recently described variant identified in Shiga-toxigenic (STEC), enterohemorrhagic (EHEC) and enteropathogenic *E. coli* (EAEC). However, three isolates were espP negative and one was nleF and nleA negative. The 24 non-O157 STEC isolates comprised of: 18 (75%) positive for stx1 -16 were stx1a and 2 were non typeable (NT) based on the primers used; 2 (8.3%) for stx1a and stx2; 2 (8.3%) for stx2; 1 (4.2%) for stx1 NT and stx2a; and 1 (4.2%) for stx2a. Only one person required hospitalization for rehydration and analgesia. Patient age in this group ranged from 6 months to 83 years. The most prevalent serotypes were O26:H11/HNM (17%) and O121:H19 (17%) followed by O111:HNM/NT (13%), O103:H25 (13%), O145:HNM (8%) and O5:HNM (8%). The virulence gene profiles vary within and between the serotypes.

**Conclusions:** The majority of the O157 STEC isolates were positive for all the virulence markers tested in this study in contrast to the non-O157 STEC. No difference was observed between the genetic profiles of isolates in the HUS and the non HUS cases. In the non-O157 STEC group, the profiles of the different serotypes varied but no conclusion can be drawn based on the limited number of cases studied.

**P-083**

**Biofilm Characteristics of the Recent German O104:H4 Outbreak Strain and Related Pathogenic Escherichia coli**

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**Introduction and Objectives:** In early summer 2011 there has been an outbreak of EHEC O104:H4 with >4000 patients infected, >20% developing hemolytic uremic syndrome (HUS), and 50 cases of death (1). Rapid genome sequencing revealed the outbreak strain to be a novel hybrid of EAEC (which contributed about 90% of the genome) and classical EHEC (which contributed the shiga toxin encoding stx2, which is responsible for HUS, as well as additional virulence genes) (3). It has been speculated that the unusually high incidence of HUS may be due to increased adherence to intestinal epithelium, which might promote systemic uptake of Shiga toxin (1). While adhesin and matrix substances of bacterial biofilms are highly species-specific, expression of these components is almost ubiquitously activated by the second messenger cyclic-di-GMP. This signaling molecule is produced by diguanylate cyclases (DGC, carrying GGDEF domains) and is degraded by specific phosphodiesterases (PDE, with EAL domains), which usually occur in multiples (E. coli K-12 strains contain 29 GGDEF/EAL domains) (2). In this study we compared a series of biofilm-associated properties for the O104:H4 outbreak strain, the closely related HUSEC041 and 55989 EAEC strains, the classical EHEC strain EDL933 and E. coli K-12 (strain W3110). In particular, we analyzed biofilm architecture under different surface growth conditions, the formation of relevant fimbiare and matrix components and the expression of corresponding regulatory genes. Moreover, the outbreak strain exhibited a high mutation rate leading to the frequent occurrence of lineages with altered adhesive and biofilm potential.

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P-083
Bioinformatic Analysis of Cyclic-di-GMP Signaling in the Recent German O104:H4 Outbreak Stain and Related Pathogenic Escherichia coli
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Introduction and Objectives: In early summer 2011 there has been an outbreak of EHEC O104:H4 with >4000 patients infected, >20% developing hemolytic uremic syndrome (HUS), and 50 cases of death (1). Rapid genome sequencing revealed the outbreak strain to be a novel hybrid of EAEC (which contributed about 90% of the genome) and classical EHEC (which contributed the shiga toxin-encoding stx2, which is responsible for HUS, as well as additional virulence genes) (3). It has been speculated that the unusually high incidence of HUS may be due to increased adherence to intestinal epithelium, which might promote systemic uptake of Shiga toxin (1). While adhesins and matrix substances of bacterial biofilms are highly species-specific, expression of these components is almost ubiquitously activated by the second messenger cyclic-di-GMP. This signaling molecule is produced by diguanylate cyclases (DGC, carrying GGDEF domains) and is degraded by specific phosphodiesterases (PDE, with EAL domains), which usually occur in multiples (E. coli K-12 strains contain 29 GGDEF/EAL genes) (2). In order to get more insight into their adhesive potential, we performed a comparative genomic analysis of GGDEF/EAL genes of the O104:H4 outbreak strain, the closely related HUSEC041 and 55989 EAEC strains, the classical EHEC strain EDL933 and E. coli K-12. We found that the outbreak strain, HUSEC041 and 55989 possess two additional GGDEF genes encoding active DGCs, while EDL933 features an additional EAL gene encoding an active PDE. Moreover, several small variations in GGDEF/EAL genes and other genes related to c-di-GMP signaling were detected. Our data are consistent with the very different surface biofilm morphologies that we observe for these strains and indicates a particularly high adhesion potential in E. coli O104:H4.

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P-084
Factors Controlling the Transcriptional Expression of Long Polar Fimbriae 2 (Lpf2) of Enterohemorrhagic Escherichia coli O157:H7
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Introduction and Objectives: To study the transcriptional regulatory mechanism controlling the expression of Lpf2 in enterohemorrhagic E. coli (EHEC) O157:H7.

Material and Methods: A primer extension assay was conducted to obtain the cDNA, using 10 μg of EHECwt RNA and a 32P-labeled probe located 52 bp downstream of the translational start site of lpfA2. We simultaneously carried out a DNA sequencing reaction using the dideoxy method and a DNA template corresponding to a region of 401 bp located at the 5'-upstream of the ATG of lpfA2. RT-PCR and real-time RT-PCR assays were performed using RNA extracted from EHECwt strain EDL933 and EHEC fur mutant grown in DMEM, pH 6.5 and 7.2 at 37°C and OD600 of 0.8, 1.0 and 1.2, in presence or absence of iron. We performed cDNA synthesis with an oligonucleotide located 33 bp upstream of the lpfA2 stop codon and PCR was performed with two oligonucleotides that amplified a 135 bp fragment of the lpfA2 gene. As a control for expression of the transcript, we used 16S RNA under the different conditions. The lpfA2 and fur genes were cloned into the pExt-6His expression vector. The Lpf2-6His and Fur-6His proteins were purified using nickel columnand used to raised antibodies in rabbits. Western blot analysis was carried out with total cell extracts of EHEC wt, EHEC lpfA2 mutant (lpfA2::tet) and EHECwt containing the operon lpf2 to measure the effect of temperature, culture medium and iron on lpfA2 expression levels.

Results: We found the transcriptional start site located at -171 bp of the translation start site of lpfA2 gene in EHEC wt strains 86-24 and EDL933, using DMEM at 37°C, pH 6.5 and 7.2 and OD600 of 1.2. RT-PCR showed that the lpfA2 transcript level was higher in DMEM at 37°C at pH 6.5 and OD600 of 1.0 and 1.2, and decreased under iron depletion but increased in the fur mutant. The lpfA2 transcript at OD600 of 1.0 and pH 6.5 was four times greater than that observed at pH 7.2.

Conclusions: This result indicated that lpf2 expression is induced at pH 6.5 and during early exponential phase and suggested a complex regulation by iron. The increase in lpfA2 transcript expression levels in the absence of the regulator Fur suggested the role of this protein in the repression of the lpf2 operon. The level of Lpf2A expression in total extracts was higher in MacConkey than LB and at 25 than 37°C.

P-087
Characterization and Distribution of a Pathogenicity Island Vehiculating the SubAB2 Variant of Subtilase Cytotoxin
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Introduction and Objectives: Subtilase (SubA) cytotoxin is produced by some VTEC strains lacking the locus of enterocyte effacement (LEE). The prototype SubAB genes (subAB) are located on a virulence plasmid together with saa gene, encoding an autogglutinating adhesive. We recently identified a new allelic variant of the subAB genes (subAB2), produced by two VT-negative E. coli strains and harbourered on a putative pathogenicity island (PAI) located on the chromosome and vehiculating also tia gene, encoding an invasion determinant of Enterotoxigenic E. coli (ETEC). It has been shown that subAB is rarely found in VTEC isolated from cattle, while it seems to be more common in strains from small ruminants,
with subAB2 as the predominant variant. This work aimed at characterising the putative PAI vehiculating subAB2 and at evaluating the prevalence of the two variants in VTEC strains isolated from human and animal sources.

**Material and Methods:** The subAB2 flanking regions were characterized by chromosome walking. A genomic library was prepared from a positive strain and the clones obtained were sequenced and assembled in contigs with the Kodon software (Applied Maths, Belgium). The presence of the two subAB variants was investigated in 219 human *E. coli* isolates including 38 ETEC, 26 Enteropathogenic *E. coli*, 20 Enteropathogenic *E. coli*, 15 Enteroinvasive *E. coli* and 120 VTEC from the culture collections of the Staten Serum Institut (Copenhagen, DK) and in 126 LEE-negative VTEC strains isolated from 48 sheep from four farms in Extremadura (Cáceres, ES). The screening was performed by using PCR specifically deployed for each of the alleles.

**Results:** The subAB2 variant is located in an 8 kb PAI (SEP-PAI). Alongside with the subAB2 and tia genes it harbours the Shigella gene shiA and encodes an integrase and a hydrolase. The screening of the human isolates confirmed the association of subAB with LEE-negative VTEC only, where subAB was present in 60% of the tested VTEC strains. Additionally, 106 strains belonging to the positive serogroups were tested and 95.3% were subAB-positive suggesting a clonal acquisition of this determinant. All the positive strains harboured the subAB2 variant and tia gene, indicating the presence of the SEP-PAI. Among the animal strains, 86.5% were subAB-positive, with 78.9% of them possessing the SEP-PAI, as indicated by the presence subAB2 and tia.

**Conclusions:** The characterisation of the genomic region harbouring the subAB2 genes confirmed that this variant is vehiculated by a PAI that we termed SEP-PAI. Moreover, we showed that the subAB variant harboured by SEP-PAI is the most common variant in the LEE-negative VTEC isolated from human cases of diarrhoea as well as from sheep in Spain. These findings indicate that sheep could be a reservoir for subAB-producing strains, suggesting the possible zoonotic transmission of these pathogens to humans.

**P-088**
**Biological Fitness Impact of Antibiotic Resistance and Virulence Gene Acquisition on Shiga Toxin-Producing *Escherichia coli* Strains**

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**Introduction and Objectives:** The rapid spread of virulence genes and antimicrobial resistance is a consequence of intra- and interspecies gene transfer. Antimicrobial resistance is sometimes associated with a biological fitness cost, in other words, a reduction in growth rate and bacterial fitness cost. In the present study the fitness impact of plasmid-encoded antibiotic resistance (tetA, tetB, strA) and virulence (ehxA, spates, toxB) genes from O26:H11 Shiga toxin-producing *Escherichia coli* (STEC) human isolates on *E. coli* HS strains and K-12 MG1655 was evaluated.

**Material and Methods:** Plasmid incompatibility was determined by PCR based replicon typing. Disc diffusion and PCR were used to determine antimicrobial susceptibility and to identify antimicrobial resistance and/or virulence genes, respectively. Plasmids were extracted by alkaline lysis. The fitness impact of plasmids encoding virulence and antimicrobial resistance upon bacterial hosts was determined by pair-wise growth competition in Davis minimal medium over 40 generations.

**Results:** The plasmid profile analysis showed that STEC strains had two to six bands of high and low molecular weight plasmids belonging to the P, F, FepB, KB/K and/or K/B/K/O incompatibility groups. Competition experiments showed that acquisition of two separate plasmids carrying tetA or tetB/strA from *E. coli* STEC by *E. coli* HS was associated with a low biological cost (2.2 ± 1.9% and 2.1 ± 1.3%). Acquisition of plasmid carrying tetB/strA from STEC by *E. coli* MG1655 revealed the absence of biological cost (0.2 ± 1.3%).

**Conclusions:** The results obtained at the present moment indicate that the fitness cost imposed on different host bacterial carrying only resistance genes from STEC strains was low or non-existent, suggesting that there was little or no effect on the growth of the recipient strains. This in turn suggests that there are few biological barriers to the spread of virulence and resistance genes from STEC to other *E. coli* strains.

**P-089**
**The Gam/CII Region of the ϕ8 vtx2-Converting Phage of *Escherichia coli* O157 (VTEC O157) Influences the Regulation of the LEE1 Promoter**

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**Introduction and Objectives:** Some phage types (PT) of VTEC O157, such as PT8, are associated with severe human infections, while others, such as PT 21, seem to be restricted cattle. We identified in PT8 strains a vtx2-phage, termed ϕ8, which is also present in other VTEC O157 PTs associated with human disease and in PT21/28, a PT associated to super-shedding in cattle. Since vtx-phages can influence the regulation of the LEE locus, which governs the induction of the attaching and effacing (A/E) lesion, we investigated the possible effects of ϕ8 on the transcription of LEE-encoded genes.

**Material and Methods:** The EspD protein is secreted via the LEE type three-secretion system (TTSS) and can be considered as an indicator of the TTSS functionality. The amount of secreted EspD was evaluated by Western blotting in strains belonging to PT8, PT21/28 and PT21. The regulation of the TTSS coding genes was investigated by co-transforming the K12 strain JM109 with the plasmids pAJR71, containing the Green Fluorescent Protein gene controlled by the LEE1 promoter, and pGEM, containing the gam/CII region of ϕ8, or the same region from another vtx2-phage present in PT21 strains.

**Results:** Western blotting showed that strains belonging to PT8 and PT21/28 secreted amounts of EspD lower than those secreted by the PT21 isolates. Consistently, the co-trasfection of JM109 cells with the pAJR71 and the pGEM containing the gam/CII region of ϕ8 showed a significant repression of the LEE1 promoter, compared with the same system transformed with the gam/CII region of the PT21 phage or with pAJR71 alone.

**Conclusions:** In conclusion, we observed that VTEC O157 PTs associated with human disease or to the super-shedding phenomenon in cattle secrete lower amounts of EspD than strains belonging to PT21, a PT usually restricted to cattle. Since EspD is secreted via TTSS, which plays a key role in the A/E lesion, its lower secretion may hamper the functionality of TTSS. Given our previous observations of the presence of ϕ8 in a high proportion of VTEC O157 associated with either super-shedding or severe human disease, we investigated its effect on the regulation of the TTSS genes carried by the LEE1 operon. A repressive effect on the LEE1 promoter was actually exerted by the gam/CII region of ϕ8, but not by the same
Introduction and Objectives: Shiga toxin (Stx)-producing *Escherichia coli* (STEC) play an important role as pathogens in humans and pigs. Stx2 and, to a minor extent, Stx1 of STEC strains cause severe kidney and central nervous system complications in humans, whereas Stx2e represents the major virulence factor responsible for the pig edema disease. Here we present a comprehensive comparative glycosphingolipid (GSL) receptor binding study of Stx1 and Stx2 derived from human STEC isolates, and Stx2e derived from porcine as well as from human isolates.

Material and Methods: GSL-Stx-interaction was analyzed in thin-layer chromatography (TLC) overlay assays using Stx-containing bacterial culture supernatants from STEC of various serotypes. TLC-separated GSLs were overlayed with supernatants and bound toxins were detected directly on the TLC plate using anti-Stx1 or anti-Stx2 antibodies followed by incubation with the corresponding alkaline phosphatase (AP) conjugated secondary antibodies and indolylphosphatase as the substrate.

Results: All Stx1-containing supernatants exhibited strong and moderate toxin binding towards globotriaosylceramide (Gb3Cer) and globotetraosylceramide (Gb4Cer), respectively, suggesting both GSLs as almost equivalent receptor sites. In contrast, all Stx2-containing supernatants showed a predominant interaction with Gb3Cer and low binding to Gb4Cer. All supernatants containing Stx2e revealed enhanced binding to Gb4Cer as compared to Gb3Cer. Thus, Stx2e, though structurally closely related to Stx2, exhibited greater similarity to the recognition profile of Stx1 than of Stx2. To further investigate the possible interaction of the toxins with extended globo-series GSL structures, we probed a GSL with a pentahexose core structure regardless if derived from human or porcine isolates, exhibited strong binding towards this prolonged globo-series GSL, which is increased risk of exposure for humans could explain the high prevalence of phage \( \Phi8 \)-positive strains among VTEC O157 from severe human infections.

Conclusion: The presence of phage \( \Phi8 \) might influence the mechanisms involved in cattle colonization by of VTEC O157 by down-regulating the TTSS-coding genes. The possible involvement of the phage in favouring the super-shedding phenomenon and the consequent increased risk of exposure for humans could explain the high prevalence of \( \Phi8 \)-positive strains among VTEC O157 from severe human infections.
In our settings, seems to be uncommon. This protein was termed STEC autotransporter contributing to biofilm formation (Sab). This study aimed at investigating the presence of Sab among the several serotypes investigated. The prevalence and distribution of Sab among STEC isolates in Brazil. The prevalence and distribution of Sab among STEC isolates were slightly higher among STEC isolates in Brazil. The prevalence and distribution of Sab among STEC isolates were slightly higher among STEC isolates in Brazil. The prevalence and distribution of Sab among STEC isolates in Brazil.

**Material and Methods:** Polymerase chain reactions (PCR) using primers LHO147-f / LHO147-r, and strain EH41 as control, were performed to search for Sab. Ability to form biofilm was tested in all O113:H21 STEC isolates, and in the subpopulations of STEC isolates by using the polystyrene microplate assays at 28 or 37°C, for 48 h. A correlation between the presence of Sab and other STEC virulence associated genes was also analyzed.

**Results:** Fourteen (11%) of the 133 strains tested were positive for Sab. These strains, all from non-human sources, belonged to a diversity of serotypes (O3:H19, O5:HNM, O96:H19, O105:H18, O116:H21, O118:H16, O178:H19, ONT:H7, ONT:H8 and ONT:H19). Biofilm formation was observed in 4 (28%) of the 14 Sab+ isolates. None of the O113:H21 strains were Sab+, however 17 (50%) of them produced biofilm under the tested conditions. Analysis of virulence associated genes showed that 71% of Sab+ isolates also carried iha, saa, ehxA and subAB.

**Conclusions:** This is the first report about the occurrence of Sab among STEC isolates in Brazil. The prevalence and distribution of Sab among the several serotypes investigated were slightly higher than previously described. Despite the original description of Sab in STEC O113:H21, its occurrence in strains of this specific serotype, in our settings, seems to be uncommon.

**P-094**

**LEE-Negative Shiga Toxin-Producing *Escherichia coli* Strains Associated with Human Disease in Argentina**

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**Introduction and Objectives:** In Argentina, Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is the prevalent serotype associated with outbreaks and sporadic cases of diarrhea (D), bloody diarrhea (BD) and hemolytic uremic syndrome (HUS). In addition, non-O157 STEC serotypes, described as highly virulent, are identified mainly as O145:NM, O26:H11, O103:H2, and O121:H19. However, LEE-negative strains with different virulence profiles are detected with a low frequency, associated to severe human disease. The aim of this study was to characterize LEE-negative STEC strains isolated from human disease in Argentina.

**Material and Methods:** Between 2000 and 2010, a total of 60 LEE-negative STEC strains were isolated from human disease: HUS (32), D (20), BD (7), and septicaemia (1). STEC strains were phenotypically and genotypically characterized. The stx-genotyping was performed by PCR-RFLP, including the stx<sub>2</sub>activatable gene detection.
immunogold electron microscopy and western blotting in bacteria cultured with the colonic cell line. However, deletion of csgA (encoding the putative major subunit of curli) did not appear to affect intestinal colonization. Inclusion of lpfA alleles in the mPCR assay permitted differentiation of EHEC1 and EPEC2 groups, as well as EHEC1 and EHEC2. This methodology resulted in a clinical sensitivity and specificity of 91% and 84%, respectively, when analyzing clinical strains from Argentina.

Conclusions: Further work is needed to more fully examine the link between curli and Lpf production and the specific contribution of each one during colonization and persistence. The use of lpfA alleles in the multiplex PCR assay allowed us to discriminate different classes of pathogenic E. coli strains, suggesting this can be used for diagnosis of STEC and EPEC infections.

P-096
Detection of Integrons Class 1 and Class 2 in VTEC Strains Isolated from Pigs
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Introduction and Objectives: In the last 10 years, approximately 500 HUS new annual cases were reported in Argentina, with an incidence of 17/100 000 children under five years old. The production of VT1, VT2 and/or their variants is the primary virulence trait responsible for human disease. VT2e is typically associated with pig edema disease and has been detected only rarely in VTEC of human origin. Some studies have shown that VTEC serotypes have developed resistance to antibiotics used in human and veterinary medicine. Humans may obtain antibiotic-resistant E. coli by contact with animals, foods, or environment. The widespread use of antibiotics creates a reservoir of resistant bacteria carrying antibiotic resistance genes. Integrons contain the genetic determinants of the components of a site-specific recombination system which recognize and capture genes. Integrons include a gene for an integrase (int), an adjacent recombination site (attl), and a strong promoter/s that ensure expression of the integrated cassette. The aim of this study was to detect integrons in VTEC strains with antibiotic multiresistance.

Material and Methods: Twenty-one VTEC strains isolated from pig faeces from 10 farms (named A to J) from Argentina were analysed to detect integrons (int1 and int2) by PCR.

Table 1: Results

| Farm | Toxin | subtype | int1 | int2 | Antibiotic resistance |
|------|-------|---------|------|------|-----------------------|
| A    | vt2e  | int1    | S-AMP-TET-CMP-TMP/SMX-DOX-FLOR |     |                       |
| B    | vt2e  | int2    | S-T-CMP-TMP/SMX-DOX-FLOR |     |                       |
| C    | vt2e  | int2    | S-T-CMP-TMT/SMX-DOX-FLOR |     |                       |
| D    | vt2e  | int2    | TET-DOX-FLOR-CIP-NAL |     |                       |
| E    | vt2e  | int2    | S-T-CMP-TMP/SMX-DOX-FLOR |     |                       |
| F    | vt2e  | int2    | S-T-CMP-TMP/SMX-DOX-FLOR |     |                       |
| G    | vt2e  | int2    | S-T-CMP-TMT/SMX-DOX-FLOR |     |                       |

CIP, Ciprofloxacin; AMP, Ampicillin; TMS, Trimethoprim/Sul-famethaxazole-DOX, Doxycycline; CMP, Choramphenicol; FLOR, Florfenicol; TET, Tetracycline; NAL, Nalidix acid; S, Streptomycin.

Results: Out of 21 analysed strains, 8 (38%) carried integrons encoding genes. These strains belonged to four different farms. The results of PCR and antibiotic resistance are shown in Table 1.

Conclusions: The use of antibiotics in animal production systems has determined that bacteria could develop resistance mechanisms originating strains with risk for human if entering a food chain. Integrons are not only associated with resistance to antibiotics, but also with the horizontal transference of resistance genes. Some studies have informed of the presence of integrons in Enterobacteriaceae and Escherichia coli in samples isolated from pigs, not being registered data in VTEC strains so far. In this study integrons class 1 and class 2 were detected in VTEC strains isolated from pigs showing that inadequate use of antibiotic as therapeutic agents or growing promoter in veterinary, implies a risk for public health because the acquisition and the horizontal transference of integrons among strains.

P-097
Serotypes and Virulence Genes of Shiga Toxin-Producing Escherichia coli Isolated from Cattle in Brazil
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Introduction and Objectives: Cattle are the main animal reservoir of Shiga toxin-producing Escherichia coli (STEC). The aim of this study was to characterize the serological, phenotypic and virulence genes of STEC isolated from bovine stools at Rio de Janeiro State, Brazil.

Material and Methods: Stool samples of 2402 healthy animals were examined by multiplex PCR (stx1, 2 and eae genes; China et al., 1996). The stx-positive stool samples were then tested for rfb O157 gene by PCR (Paton and Paton, 1998b). E. coli O157 was isolated by immunomagnetic separation followed by plating onto Sorbitol-MacConkey agar plus tellurite (2.5 mg/L) and cefixime (0.05 mg/L). The other STEC serotypes were isolated by colony hybridisation with a radiolabeled stx probe (Feinberg and Vogelstein, 1984) using the PCR product of E. coli EDL933 strain as a probe (Paton et al., 1993). E. coli serotypes were identified with O1 to O181 and H1 to H56 antisera. The virulence genes EHEC-hlyA (Paton and Paton, 1998b), espP (Brunder et al., 1999), saa (Paton and Paton, 2002), iha (Schmidt et al., 2001) and astA (Yamamoto, 2000) were detected by PCR. The haemolytic activity was tested according Beutin et al. (1995) and production of Stx was investigated on Vero cells (Smith and Scotland, 1993). Cell-adherence assay (Cavrioti et al., 1979) and fluorescence actin staining (FAS) test (Knutton et al., 1989) were both performed with HEP-2 and Caco-2 cells after 3 h-infection. A negative cell-adhesion or FAS test was repeated after 6 h-infection.

Results: A total of 1562 (65%) animals were positive for stx genes. E. coli O157 strains were isolated from 27 fecal samples positive for rfb O157 gene and eight isolates (42.1%) were identified as STEC (stx-positive). Nine hundred and ninety-two non-O157 STEC strains from 33 different serotypes were isolated. All STEC strains were able to induce a cytotoxic effect on Vero cells. STEC serotypes were ascribed to two groups – group I, that occur in human disease (73 strains) and group II, not yet described in human disease (27 strains). The stx2 genotype was significantly associated with O157:H7 serotype. However, this genotype occurred among strains from different serotypes in both groups. Most strains in groups I and II, were intimin-negative. Concerning the other virulence genes investigated we did not find differences between serotypes or between O157 and non-O157 strains, with exception of astA, signifi-
cantly associated with STEC O157:H7. Among the strains that harbored the hlyA gene, 58% expressed the haemolytic phenotype. **Conclusions:** This study shows that cattle from farms of distinct counties at the Rio de Janeiro State, Brazil, is an important reservoir of STEC, hosting a diversity of serotypes, most of them carrying genes linked to human disease. These results emphasize the need for control measures in the production of food of bovine origin.

P-098
Atypical Virulence Factors in Shiga Toxin-Producing Escherichia coli Isolated from Sick Calves
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**Introduction and Objectives:** Shiga toxin-producing *Escherichia coli* (STEC) is a zoonotic pathogen of global importance, whose main reservoir is the intestinal tract of cattle. In addition to Shiga toxins (Stx), STEC strains often possess other virulence determinants, as the products of Locus of Enteroceptor Effacement (LEE). Not much information is available about the presence of virulence factors characteristic of *E. coli* belonging to other pathotypes. The aim of this study was to determine the presence of virulence factors characteristics of enterotoxigenic, septicemic or extraintestinal pathogenic *E. coli* in STEC isolated from calves with diarrhea or septicemia.

**Material and Methods:** The *E. coli* isolates were recovered from sick calves under 3 months old during the years 2007–2011. Stx (stx1 and stx2) positive isolates were analyzed by three multiplex PCR for the detection of the genes of adhesins (F5 (F5), F41 (F41), Intimin (eae), Afa-8 (afaEVIII), S (sfa), F17 (f17), and P (papC)), toxins (Cytolethal Distending Toxin Types III (cdtBIII) and IV (cdtBIV)), Cytotoxic Necrotizing Factors Types 1 (cnf1) and 2 (cnf2), and Heat-stable Enterotoxin (Sta) and Aerobactin (iucD).

**Results:** Of the 51 isolates, 44 (86%) were stx1 and 7 (14%), stx2. We have not found sta, cnf1-cnf2 or the stx1-stx2 combination. With respect to adhesins, the gene of Intimin was detected in 45 isolates (88%). The gene of P fimbria was found in seven isolates stx1 (14%), and was followed by f17 in four isolates (8%), and afaEVIII, found in three isolates (6%). We did not detect f5or f41. Adhesins Afa-8 are typical of cattle, while Intimin, F17 and P are shared by human isolates. We found that 22% STEC had one or more of these adhesins. The gene of the aerobactin was detected in 22 (43%) stx1 isolates. One (2%) and 2 (4%) stx1 isolates carried cdtBIII and cdtBIV, respectively. These factors are common in extraintestinal *E. coli* strains.

**Conclusions:** The associations of various virulence factors showed great heterogeneity. Most of the isolates carried two factors of virulence (25, 49%), being the most frequent, stx1-eae and stx2-eae. Those isolates with three factors (19, 37%) showed some heterogeneity, with the majority carrying stx1-eae-iucD. Seven (13%) isolates had several combinations of four virulence factors. Some of the genes detected (papC) have been identified as belonging to chromosomal pathogenicity islands or plasmids (cdtBIII, f17, iucD) or other newly acquired mobile genetic elements. The presence of unusual repertoire of virulence genes in STEC strains of bovine origin may contribute to pathogenicity, showing a widespread tissue tropism, as well as increased cytotoxicity. The enhanced virulence potential for humans and animals and their relationship to known clones should be investigated.

P-101
Binding Properties of Immunoglobulin-Binding Protein EibG, a Novel Trimeric Autotransporter Adhesin, from Shiga Toxin-Producing *Escherichia coli*
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**Introduction and Objectives:** Seven related immunoglobulin (Ig)-binding proteins namely EibA, C, D, E, F, G and H are expressed by different Shiga toxin-producing *Escherichia coli* (STEC) strains. These proteins share the capability to bind IgG and IgA by nonimmune manner, allowing for evasion of host immune response. Moreover, EibG is involved in bacterial adherence to host intestinal epithelial cells. Depending on different eibG-alleles, eibG-positive strains show variable chain-like adherence pattern (CLAP) on epithelial cells. EibD and EibG were recently discovered as trimeric autotransporter adhesins (TAAs). The aim of this study was to get deeper insight in the binding properties of different EibG subtypes and their membrane organisation.

**Material and Methods:** Detection of eibG-positive strains was performed with the inner eibG-fragment by means of PCR techniques. For the sequencing of eibG-gene ABI Prism BigDye Terminator v3.1 ready reaction cycle sequencing kit was used. CLAP phenotyping of eibG-positive strains and expression of EibG on bacteria surfaces were investigated in adhesion assays and by immunofluorescence microscopy, respectively. Distribution of EibG within inner and outer membranes of *E. coli* was analysed by sucrose density gradient centrifugation. Interaction of EibG-subtypes to IgG of different vertebrate species was determined by ELISA under variable conditions.

**Results:** Within a large STEC collection 36 eibG-positive strains were detected only in eae-negative strains. Sequencing of entire eibG-gene resulted in 21 different eibG-alleles, which were allocated to three subtypes eibG-α, -β and -γ and confirmed by peptide mapping employing matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Adhesion assays with human and bovine epithelial cells demonstrated different CLAP-phenotypes depending on eibG-subtypes. Analysis of outer and inner membranes of eibG-positive *E. coli* gave evidence for occurrence of EibG oligomers in the outer membrane and verified its identification as TAA. EibG showed high affinity to human, bovine, sheep, goat and rabbit IgG and also to chicken IgY, indicating its potential zoonotic risk. Binding studies of EibG with glycosphingolipids (GLSs) of animal cells by thin-layer chromatography overlay assay techniques revealed most likely unspecific EibG-GLS interaction.

**Conclusions:** Further analysis of the molecular mechanisms of the three different EibG-subtypes should help to better understand their biological impact on nonimmune reactions and interaction with target epithelial cells. This work was supported by grants from the Federal Ministry of Education and Research Network Zoonoses and by grants from the Interdisciplinary Center of Clinical Research (IZKF) Münster.

P-103
Protective Effects of Lactic Acid Bacteria on Enterohemorrhagic *Escherichia coli* O157-Infected Cultured Epithelial Cells
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**Introduction and Objectives:** In this study, we investigated enterohemorrhagic *E. coli* (EHEC) O157 infection in a model with HT29 epithelial cells. Protective effects of certain lactic acid bacterial (LAB) strains on this process were analyzed in co-infection assays. Among other host signal transduction pathways, EHEC induces activation of MAP kinases, and nuclear transcription factors (NF-kB and AP-1) to induce the production and secretion of cytokines or chemokines, for example, Interleukin-8 (IL-8). IL-8 serves as a potent chemotaxant that delivers neutrophils to the site of bacterial infection. A high IL-8 level is deemed to be an indicator of a high risk for developing a typical haemolytic uremic syndrome. Toll-like receptors (TLR) are components of the human innate immune system and recognize bacterial surface structures.

**Materials and Methods:** HT29 cells were stimulated in co-infection assays with the EHEC O157:H7 strain EDL933 in a multiplicity of infection (MOI) of two and with LAB in a MOI of 200. One hour after infection the cells were washed with PBS to remove the non-attached bacteria and were further incubated. After it, the cells and the culture supernatants were collected for subsequent analysis.

**Results:** Gene expression analysis showed a down regulation of the TLR4 gene after infection of the epithelial cells with EDL933. For the TLR9 gene, an up-regulation was observed. In co-infection trials, the benign bacteria did not influence the EHEC-induced TLR4 expression but some of these lower the expression of the TLR9 gene significantly. The TLR2 gene was not regulated neither after infection with EDL933, nor by co-infection with LAB. The activation of the 'nuclear factor-kappa B' (NF-kB) in HT29-cells was measured by a reporter gene assay. Thereby, co-infection assays with EDL933 and LAB-strains showed a lower activity of NF-kB than by infection with EDL933 only. No strain-specific effects could be observed. After mono-infection with EDL933, HT29 cells produced high amounts of IL-8. The co-infection of HT29-cells with certain LAB strains decreased the IL-8 production up to 60% significantly.

**Conclusions:** The results of this study show that particular LAB strains are able to reduce the pro-inflammatory response and modulate the innate immune system of EHEC-infected HT29 cells. Further studies are needed to investigate the protective effects in EHEC-infection in vivo.

**P-104 Acid Resistance and Biofilm Production Markers Among STEC strains Isolated from Foods and Cattle in Brazil**

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**Introduction and Objectives:** The interaction of pathogens with their hosts and the environment involves several strategies for adherence, colonization and survival. Among the many factors involved in this process, the ability of biofilm formation and expression of acid-resistance are particularly relevant in the context of transmission of pathogens from their reservoirs to humans generally by food vehicles. This study aimed to evaluate the expression of acid-resistance and phenotypic and genotypic biofilm markers of STEC strains isolated from foods and cattle. The profile of the strains studied was not singular and was similar to other strains but despite acid resistance and biofilm production not being exclusive characteristics of pathogenic strains, this behavior of STEC strains helps them to interact and survive in the environment and in their host.

**P-106 EDTA Triggers Induction of Shiga toxin2-encoding Phages Independently of RecA**

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**Introduction and Objectives:** Shiga toxins (Stx) are the cardinal virulence marker of STEC. The genes encoding Stx are located in the genome of lambdoid bacteriophages (Stx phages) The described mechanism that stimulates prophage induction and Stx production involves SOS response and RecA activation (e.g. upon mitomycin C or UV light treatment). Once released from the cell, Stx phages provide a mean of transduction of stx genes into new hosts. Previous studies from our group suggest that prophage induction might also occur in the absence of the SOS response, independently of RecA.

**Material and Methods:** The influence of EDTA on phage induction was assessed by Real-Time PCR using EHEC strain EDL933 and laboratory lysogens of phage 933W, as well as lysogen of RecA isogenic mutant lacking the whole recA gene. Cultures treated with 20 mM of EDTA showed Stx phage induction even in the RecA-mutant, indicating that EDTA induction is RecA-independent. Induction was not observed with the mutant when using mitomycin C. To gain insights on the RecA-independent mechanisms of induction, we evaluated mechanisms previously described for phage λ induction and three pathways for stress response of bacterial envelope affected by EDTA. Those pathways were evaluated by generating mutants where the targets genes were replaced by a tet cassette. Results indicated that none of these possibilities were contributing to Stx2 phages induction mediated by EDTA.

**Results:** It was finally demonstrated that the effect of EDTA on the Stx phage induction is due to its chelation properties. The use of another chelating agent, sodium citrate, confirmed these results by increasing Stx phage induction in the RecA-mutant. EDTA and sodium citrate have affinity for Mg2+ and Ca2+. Induction was not observed by using chelants more specific for Ca2+ or Fe3+ or Zn2+, suggesting that probably Mg2+ is the cation involved in the process. The use of AB minimal medium lacking the different cations did not reduce EDTA-mediated phage induction. However, the use of medium supplemented with cations clearly reduced the phage induction in the presence of EDTA. Our results suggest that chelations of cations from the bacterial outer membrane produces the signal that triggers Stx phage induction. Throughout the conditions evaluated, the pH value had a decisive role in Stx2 phage induction.

**Conclusions:** Chelating agents, such as EDTA, are able to induce Stx phage and this fact is of concern due to their common use in food and pharmaceutical products. This study contributes to our understanding of the phenomenon of induction and release of Stx phages as an important factor in the pathogenicity of STEC and in the emergence of new pathogenic strains.
**P-108**

**Enterohemorrhagic Escherichia coli O157:H7 Shiga Toxins Inhibit Interferon-Gamma Mediated Signal Transducer and Activator of Transcription-1 Phosphorylation**

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**Introduction and Objectives:** Enterohemorrhagic Escherichia coli (EHEC) serotype O157:H7 is a foodborne pathogen that causes significant morbidity and mortality in developing and industrialized nations. EHEC infection of host epithelial cells is capable of inhibiting the interferon gamma (IFN-γ) pro-inflammatory pathway through the inhibition of Stat-1 phosphorylation, which is important for host defense against microbial pathogens. The aim of this study was to determine the bacterial factors involved in the inhibition of Stat-1 tyrosine phosphorylation.

**Material and Methods:** Non-polarized and polarized human epithelial cells were challenged directly with either EHEC or bacterial culture supernatants, stimulated with IFN-γ, and then protein extracts analyzed by immunoblotting. 2D-Difference Gel Electrophoresis was also used to compare the secreted proteins in culture supernatants from EHEC and EPEC.

**Results:** The data showed that IFN-γ mediated Stat-1 phosphorylation was inhibited by EHEC secreted proteins and does not require bacterial contact with host cells. Using 2D-Difference Gel Electrophoresis, EHEC Shiga toxins were identified as candidate inhibitory factors. EHEC Shiga toxin mutants were then generated to confirm their ability to subvert IFN-γ-mediated cell activation.

**Conclusions:** We conclude that E. coli-derived Shiga toxins represent a novel mechanism by which EHEC evades the host immune system.

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**P-109**

**Effect of Coiled-Coil Peptides on the in vitro Function of TTSS of EHEC O157:H7 and Citrobacter rodentium and on the C. rodentium Pathogenicity in Mice**

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**Introduction and Objectives:** Many animal and human pathogenic Gram-negative bacteria such as Salmonella, Yersinia, Enterohemorrhagic Escherichia coli (EHEC) and Enteropathogenic Escherichia coli (EPEC) possess a type III secretion system (TTSS) that is used to deliver virulence proteins directly into the host cell. Recent evidence suggested that Coil A and Coil B, two synthetic peptides corresponding to coiled-coil domains of the translocator protein EspA, were effective in inhibiting the action of TTSS from entero-pathogenic E. coli (EPEC).

**Results:** In the current study, the action of these coiled-coil peptides on the TTSS of EHEC O157:H7 and C. rodentium was examined. Coil A and Coil B showed to be effective in reducing the red blood cell (RBC) lysis mediated by EHEC O157:H7 and the in vitro secretion of translocator proteins EspB and EspD by EHEC O157:H7 and EspD by C. rodentium. Treatment of mice with Coil A and Coil B peptides prevented colon damage when the animals were inoculated with C. rodentium. Colon samples of the non-treated group showed areas with loss of superficial epithelium, damaged cells and endoluminal mononuclear inflammatory infiltrate, consistent with histological lesion induced by C. rodentium, whereas mice treated with the synthetic peptides kept normal the surface epithelium showing a similar structure as the uninfected control group.

**Conclusions:** These encouraging results prompted us to test coiled-coil peptides as treatment or vaccines in other models of bacterial infections.

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**P-114**

**Effect of the Human Intestinal Microbiota and Bacteroides thetaiotaomicron on Escherichia coli O157:H7 Transcriptome**

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**Introduction and Objectives:** Enterohemorrhagic Escherichia coli (EHEC) O157:H7 is a foodborne pathogen that colonizes the colonic epithelium and causes hemorrhagic colitis and hemolytic-uremic syndrome. The human intestinal tract harbors a complex consortium of bacterial species. EHEC encounters many challenges that must be overcome for colonization to occur successfully. The human intestinal microbiota has been shown to inhibit Shiga toxin II production. B. thetaiotaominc, a predominant gut symbiont, has been identified as a potential species responsible for stx2 repression. In this study, we examined the effect of a complex human microbiota and B. thetaiotaominc on E. coli O157:H7 transcriptome by DNA microarrays and qRT-PCR.

**Material and Methods:** Germfree rats were used for the cultivation of the human fecal microbiota (HFM) and B. thetaiotaominc. Gene expression of the EHEC strain EDL933 was assessed in cecal contents of rats harbouring the HFM or B. thetaiotaominc and in cecal content of germfree rats (control condition). Briefly, 15 days post-colonization of rats, cecal contents were harvested, filtered, supplemented in nutrients and adjusted to pH 7. The EHEC strain EDL933 was cultivated for 6 h in partially anaerobic conditions in the cecal contents. RNAs were extracted and converted into cDNAs. Fragmented and biotinylated cDNAs from the three conditions were hybridized onto Affymetrix GeneChip® E. coh Genome 2.0 Arrays. Quantitative RT-PCR were performed to assess the effect of B. thetaiotaominc and to validate microarrays data. Fold change values
were calculated as the ratio of gene expression values in cecal contents of colonized rats compared to those in the control condition.

**Results:** Transcriptome profiling showed that genes carried by the locus of enterocyte effacement (LEE), required for type III secretion and attaching and effacing lesions, were repressed by both HFM and \textit{B. thetaiotaomicron}. In addition, many non-LEE genes were downregulated. Genes required for utilization of glyceral, as well as many fermentable carbohydrates were downregulated by the HFM. In contrast, genes encoding the specific uptake and degradation of the short-chain fatty acid L-lactate as well as many aromatic compounds were upregulated. Moreover, there was an induction of the ethanolamine-utilization gene cluster, which has been shown to confer a competitive advantage to EHEC during growth with resident microbiota.

**Conclusions:** These results constitute an important step in the understanding of the interactions between EHEC and the resident microbiota. Comprehension of mechanisms responsible for (i) downregulation of virulence by the microbiota and (ii) strategies used by EHEC to overcome the limited nutrients availability, would lead to the development of preventive and therapeutic approaches against STEC infections.

**P-115**

**Cutting Off the Inflammatory Response: Degradation of NF\textsubscript{x}B by NleC**

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**Introduction and Objectives:** Infection with enteropathogenic and enterohaemorrhagic \textit{Escherichia coli} (EPEC and EHEC) leads to a rapid inhibition of the host inflammatory response. Recently, several groups have conducted screens in order to identify the effector proteins involved in the inhibition of inflammation. In these studies, several translocated effector proteins have been identified which inhibit different stages of the inflammatory signaling pathway. Of the proteins described, NleB and NleE inhibit the degradation of IkB and therefore the release of the transcription factor NF\textsubscript{x}B into the nucleus where it is required to activate the expression of pro-inflammatory cytokines such as IL-8. By contrast, NleH1 and 2 bind to ribosomal protein S3 (RPS3) a subunit of NF\textsubscript{x}B transcription complex, with NleH1 repressing the transcription of RPS3/NF\textsubscript{x}B dependent genes.

**Results:** Here, we show that NleC from both EPEC and EHEC possesses protease activity conferred by a zinc metalloprotease consensus motif, HExxH, which allows the protein to cleave several, if not all NF\textsubscript{k}B subunits in transfected cells as well as during EPEC infection of HeLa cells. Mutation of the metalloprotease motif abolishes cleavage of NleC target proteins, as does the addition of the zinc metalloprotease inhibitor GM6001. Furthermore, NleC binds to and co-precipitates with the most common NF\textsubscript{k}B subunits p65 and p50 and this binding is abrogated by deletion of amino acids 32–64 suggesting the binding occurs within this region of the protein.

**P-116**

**Novel Sequence Variation Affects GP Ib\textsubscript{x} in Typical HUS Patient**

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**Introduction and Objectives:** Hemolytic uremic syndrome (HUS) is one of the major causes of renal failure in children. In most cases the disease is caused by infection with \textit{Shiga} toxin-producing \textit{Escherichia coli} (STEC) and is preceded by diarrhea (typical HUS). Only in 15% of cases the infection leads to HUS. Genetic predisposition of a patient to develop typical HUS after STEC infection might play a role, but very few reports on this subject are available so far. Here we describe a novel heterozygous missense sequence variation in the GP1BA gene encoding platelet-receptor glycoprotein Ib\textsubscript{x} (GP Ib\textsubscript{x}) in a severely affected HUS patient.

**Material and Methods:** GP1BA was screened by means of PCR and DNA sequencing using genomic DNA of the HUS patient, 192 healthy controls were analyzed for the presence of novel sequence variation. Impact of the found missense sequence variations on the protein function was analyzed using the in silico prediction programs (SIFT and Polyphen-2) and available structural data. Recombinant GP Ib\textsubscript{x} and VWF fragments were produced, purified and their binding was analyzed using Biacore. The hematological studies included analyses of mean platelet volume, VWF multimers, platelet activation markers (CD63, PAC-1, CD62p), and ristocetin-induced platelet agglutination in the presence of 0.5, 0.6, 0.7, 0.8, 0.9 and 1.52 mg/mL of ristocetin.

**Results:** The selected variation p.Pro46Leu was not found in 192 healthy controls or reported previously. The affected amino acid residue is highly conserved and is located in the proximity to one of the two von Willebrand factor (VWF) binding sites, based on the available structural data. It is predicted as damaging by mutation analysis programs SIFT and Polyphen-2. Biacore experiments show that the p.Pro46Leu change results in a modest increase in the strength of binding of GP Ib\textsubscript{x} to VWF (Kd = 690 ± 3 nM for wild type GP Ib\textsubscript{x} versus Kd = 630 ± 2 nM for p.Pro46Leu (P < 0.001)). This increase is small in comparison to the more than 30-fold stronger binding observed by us for platelet-type von Willebrand disease mutation p.Met255Val in the similar experiment, consistently, the hemostasis analysis of patient blood after recovery from HUS shows normal values.

**Conclusions:** The described change in the conserved residue affects GP Ib\textsubscript{x} interactions with VWF in a mild gain-of-function manner and might have contributed to a prothrombotic state in the patient and the development of typical HUS.
of compounds was identified as possible candidates based on the criteria of the structure-activity studies. Two compounds, CF331 and CF341, have proven to be comparable to LED209.

**Material and Methods:** In order to test the efficacy of each compound \textit{in vitro}, quantitative polymerase chain reactions were performed on EHEC virulence genes downstream of QseC signaling as well as western blots of secreted proteins of EHEC, EspA and EspB. These proteins are required for EHEC to translocate bacterial proteins into host cells and cause attaching-effacing (AE) lesions. Compounds that pass this initial \textit{in vitro} screening are then challenged in Flourescent Actin Staining (FAS) assays. Ultimately their progression ends in administration of compound into the infant rabbit model to evaluate EHEC colonization of the intestine. Pharmacokinetic studies and excretion analysis is used to analyze the compounds biochemical effects within a rat.

**Results:** CF331 markedly reduced Qse-C dependent virulence gene expression of ler, stxA2, and eae at both 5 nM and 5 pM. At 500 and 5 nM, CF331 reduced pedestal formation. CF331 reduced virulence gene expression of ler, stxA2, and eae at both 5 nM and 5 pM and modestly reduced AE lesion formation at 5 nM.

**Conclusions:** CF331 and CF341 do not inhibit EHEC growth, however, they both inhibit the virulence of EHEC \textit{in vitro} and \textit{in vivo}. The inhibition of QseC signaling allows for a novel strategy to develop antimicrobial drugs that are not responsive or resistant to traditional antibiotics.

**P-122 EspP is a Possible Virulence Factor of Enterohemorrhagic Escherichia coli**

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**Introduction and Objectives:** The infection process of enterohemorrhagic \textit{Escherichia coli} (EHEC) is presumed to be a complex interplay of different virulence factors. One of these additional effector molecules is EspP, a plasmid-encoded extracellular serine protease. EspP belongs to the serine protease autotransporter of Enterobacteriaceae (SPATE) family and is one of the most abundant proteins in EHEC supernatants under laboratory conditions. Four different EspP subtypes (EspPz-EspPz) have been identified to date. Interestingly, highly pathogenic EHEC serotypes such as O157:H7, O26:H11/NN, O111:H8/NN or O145:H28/H28/NN exclusively harbor the highly active subtype z, whereas functional impaired subtypes are found in less-pathogenic serotypes. The expression and secretion of EspP during EHEC infection has been demonstrated by the identification of EspP-specific antibodies in sera of patients. Due to its activity against complement factors C3 and C5 and coagulation factor V it has been suggested that EspPz might interact with hemostasis and the innate immune response. However, a systematic screening of relevant substrates on proteome-wide level is still lacking.

**Material and Methods:** We screened for novel substrates of EspPz using human plasma and platelets to analyze a potential interference of EspPz with central host response mechanisms in more detail. Plasma was first depleted from albumins and immunoglobulins. Platelets were activated and their supernatant was collected. After incubation with EspPz or the inactive mutant S263A the proteins were separated using SDS-PAGE as well as 2D-PAGE. To identify novel substrates, protein spots that shifted in gel electrophoretic mobility after incubation with EspPz were digested in-gel and subjected to mass spectrometrical analysis using MALDI-TOF-MS as well as nano-ESI-MS.

**Results:** It has been shown that EspPz cleaves several physiologically relevant proteins in plasma as well as in platelets including protease inhibitors and surface adhesion molecules. Further studies will elucidate the contribution of the newly identified substrates to the role of EspPz as a possible virulence factor of EHEC.
P-124
Rapid Molecular Characterization of EHEC, Their Virulence Markers and Interactions with the Bovine Host
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Introduction and Objectives: Enterohemorrhagic Escherichia coli (EHEC), including O157 and non-O157 serotypes, are food borne pathogens linked to ruminants. Their rapid detection and molecular characterization is critical to understand the ecology of these infectious agents and to control the spread of disease. To accomplish the first objective a panel of 28 genetic markers including virulence markers, O-antigens, and select proteophages regions was designed for a high-throughput PCR system and large-scale screening for EHEC O157 and 6 non-O157 serotypes (O26, O45, O103, O111, O121, and O145). The second objective was to evaluate strain-specific duration, pattern of intermittent shedding, and shedding concentration of three known E. coli O157:H7 strains of varying ecological origin in experimentally infected cattle.

Material and Methods: A total of 64 known bacterial strains were used to verify the primers specificity using the OpenArray® System (Life Technologies, Carlsbad, CA, USA). Infection experiments were conducted using four groups of six young Holstein steers housed in a controlled indoor environment. Four infection experiments were conducted using one of three groups of six young Holstein steers housed in a controlled indoor environment. Four infection experiments were conducted using one of three E. coli O157:H7 strains (47, 1641, 2533) with individual oral inoculations of 10^6 CFU per steer. Recto-anal mucosal swabs (RAMS) and environmental samples were taken every 2 days over a 30 day period and enumerated for isolation of E. coli O157:H7 and generic E. coli. All selected isolates were analyzed using the OpenArray® System for genetic profiling based on the study panel.

Results: Specific target presence and absence data were generated for the 64 bacterial strains and 2411 experimental isolates to create distinct genetic profiles. Detection of strain 47: 34.4% of RAMS were positive in three steers of group 1 and 70% of RAMS were positive with shedding events in all six steers in group 2. Detection of 1641: 42% of RAMS were positive with two steers initially shedding and the remaining steers shedding mid-way through the sample period in group 2. Detection of strain 2533: 22% of RAMS were positive in two out of six steers in group 4. During continuous shedding events in steers, E. coli O157:H7 CFU concentrations would rise to a peak (10^8–10^9 CFU/mL) and then descend (≤10^3 CFU/mL). Environmental detection of inoculation strains in each group was 13%, 21%, 22%, and 0%, respectively, and found mainly on hides and pen floors. Genotypes of EHEC and generic E. coli were clustered and dendrograms were produced for all subtypes of E. coli isolated.

Conclusions: Variation in shedding patterns and concentrations among infection groups may be due to the ecological origin of the strain, individual steer biology, or environmental factors. The panel of markers described in this study allows for quick and efficient identification of strains of interest. The panel also proved to be a time-efficient tool to generate data for a large number of isolates from the infection experiments.

P-125
Disarming the Inflammatory Response: The Type III Effector NleB from Enteropathogenic E. coli Inhibits NF-KB Activation
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Introduction and Objectives: The subversion of host cell processes by microbial pathogens is an intrinsic part of the host-pathogen interaction. Enteropathogenic and enterohemorrhagic Escherichia coli (EPEC and EHEC) utilize a type III secretion system to deliver diverse effector proteins into host cells during infection.

Results: Previously, we found that the type III effector, NleE, from EPEC blocked activation of NF-κB by inhibiting IκB degradation and p65 nuclear translocation during infection of HeLa cells. While screening for more effectors with unknown function, we also established that NleB, encoded directly upstream from NleE, suppressed NF-κB activation. Whereas NleE inhibited both TNFα and IL-1β stimulated p65 nuclear translocation and IκB degradation, NleB inhibited the TNFα pathway only, suggesting that NleB acts at a distinct point in the signaling cascade. Neither NleE nor NleB inhibited AP-1 activation suggesting that the immunodulatory activity of the effectors was specific for NF-κB signaling. The gene encoding NleB is present in all attaching and effacing pathogens, including the mouse pathogen Citrobacter rodentium and rabbit-specific EPEC (REPEC). These proteins are also capable of inhibiting NF-κB activation and p65 translocation suggesting that the activities of the NleB family of effectors are conserved.

Conclusions: Overall our data shows that EPEC and EHEC utilise T3SS-dependent means to specifically target and suppress host inflammatory signaling pathways resulting in the exquisite control of inflammatory cytokine production.

P-126
Interplay of Sigma Factors Dictates Expression of Extreme Acid Resistance and Type III Secretion in Escherichia coli O157:h7
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Introduction and Objectives: Sigma factor 38 (σ^74), encoded by rpoS, is an important regulator of extreme acid resistance (XAR) and the locus of enterocyte effacement (LEE), encoding a type III secretion system (TTSS) in O157:H7. The goal of this work is to explore the molecular basis of σ^74-directed regulation of XAR and TTSS in O157:H7 and its dependence on σ^57 for this control.

Material and Methods: Directed and site-specific mutagenesis was employed using the λ Red recombinase-assisted approach, and QuikChange™ (Stratagene), respectively. Percent survival in acid was determined by inoculation into acidified (pH 2) minimal media with glucose (0.4%) and glutamate or arginine with sampling for plate counts. Transcript and protein levels were measured for rpoS and TTSS genes using qRT-PCR, lacZ reporter fusions and western blots.

Results: During exponential growth, mutation of rpoN increased survival in acid by both glutamate- and arginine-dependent survival.
mechanisms of XAR in a manner requiring the alternative sigma factor gene rpoS. This correlated with the upregulation of σ^d-regulated genes for glutamate (gadX/gadW) and arginine (adia/adiC)-dependent XAR systems. Mutation of rpoN did not alter rpoS mRNA stability, but increased σ^d protein stability. Furthermore, mutation of rpoN as well as the σ^d activators, ntrC and flhA led to: (I) increased survival in acid during exponential growth; (II) increased σ^d protein levels, and (III) decreased expression from the promoter lep_r which controls production of the LEE central regulator, Ler. Acid resistance of a strain expressing a mutated version of rpoN (rpoN^456C), defective in DNA-binding, was significantly reduced compared to the rpoN mutant, but was significantly higher than WT.

Conclusions: This study reveals σ^d to act on σ^d by both an active and passive regulatory mechanism. Both mechanisms increase the stability of σ^d, thus altering σ^d-dependent transcription. Active regulation is hypothesized to be dependent on the NtrCB two-component system, which communicates the nitrogen status of the cell to transcription initiation through σ^d. Passive regulation, on the other hand, is believed to involve a competition between σ^d and σ^d for endogenous RNA polymerase; inactivation of σ^d thus leading to σ^d-holoenzyme formation and increased σ^d-dependent transcription. This interaction between σ^d and σ^d represents a pathway by which O157:H7 can coordinate acid resistance and type III secretion with nitrogen status, growth rate, and various other endogenous/exogenous cues.

P-128
Enhanced Virulence of the Escherichia coli O157:H7 Spinach-Associated Outbreak Strain in Two Animal Models

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Introduction and Objectives: Shiga toxin (Stx)-producing E. coli (STEC) cause food-borne hemorrhagic colitis (HC) and, in some patients, the hemolytic uremic syndrome (HUS), a serious sequel of infection. The most commonly isolated STEC in North America belong to the O157:H7 serotype. STEC strains produce one or more Stxs: Stx1 and/or Stx2, toxins that are biologically similar but antigenically distinct. A number of Stx2 variants have been described, and include Stx2c, which is 97% homologous to Stx2a at the amino acid level. Epidemiological studies suggest that stx2a, stx2b, or stx2c and stx2e E. coli O157:H7 are associated with more severe disease in humans with STEC than with other stx alleles. A 2006 spinach-associated outbreak of O157:H7 resulted in high hospitalization and HUS rates than previous STEC outbreaks, and the isolate responsible for that outbreak, K3995, was found to contain both stx2a and stx2c. We hypothesize that the virulence of K3995 is due to that combination of stx2 alleles. In this study we compared the virulence of K3995 and an isogenic Stx2 mutant to other O157:H7 outbreak strains (86-24 stx2a or 2812 stx1c in mouse and/or rabbit models.

Material and Methods: CD-1 mice received streptomycin (str) water (5 g/L) for the duration of the study. Mice were fed 10^10 CFU by pipette, and were monitored for 21 days. Intact-commensal flora BALB/c mice were infected with 10^10 CFU by intragastric gavage; weight change and intestinal colonization (as measured by CFU/g fecal pellets) were monitored for up to 15 days. Dutch belted rabbits received 10^10 bacteria by oral gavage, and were monitored for 7 days. The isogenic toxin mutant, K3995 stx2a-cat, was constructed by Lambda Red-mediated mutagenesis.

Results: In the CD-1 str-treated mouse model, K3995 infection led to more deaths than did 86-24 (Stx1c), 40% versus 3%, respectively. In contrast, in the BALB/c model, K3995 showed similar virulence and levels of colonization as 86-24. In rabbits, oral infection with K3995 proved highly virulent. Several animals exhibited intestinal pathology and succumbed to K3995 infection while those infected with 2812 (Stx1, Stx2c) had no pathologic signs and survived. In a separate study, rabbits infected with wild-type K3995 were more highly colonized than rabbits infected with K3995 stx2c; more-tolerant rabbits infected with wild-type K3995 displayed clinical signs of disease (diarrhea), dehydration, lethargy) while none of the K3995 stx2a-cat-infected rabbits displayed such signs. These data suggest that the high virulence of K3995 correlates with the presence of the stx2 allele in that strain, although a role for Stx2c cannot be ruled out.

P-130
Protection of Piglets Against Edema Disease by Active Immunization with Recombinant Inactivated Stx2e

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Introduction and Objectives: Edema disease (ED) is an enterotoxemic disorder of weaned piglets that represents a significant threat to pig husbandry worldwide. ED is caused by host-adapted Escherichia coli strains characterized by Shiga toxin subtype 2e (Stx2e) and adhesive F18 fimbria as their major virulence factors. Since therapy is difficult, attempts to control ED focus on prophylactic measures. Active immunization with whole-cell or Stx2e toxoid vaccines has been demonstrated effective to reduce the incidence and severity of ED both in challenge experiments and in field studies. In this study, we investigated whether the protective effect of recombinant Stx2e toxoids is correlated with induction of Stx2e neutralization antibodies.

Material and Methods: Suckling piglets (n = 111) were vaccinated twice at days 9 and 28 of life either with glutaraldehyde-treated recombinant Stx2e (rStx2e_662) or with recombinant, genetically modified Stx2e (rStx2e_F18_R167Q) carrying two amino acid substitutions in the catalytic center of the Stx2e subunit. Other piglets were vaccinated with a placebo (n = 30) or served as non-vaccinated controls (n = 56). All piglets were weaned at day 35, challenged intravenously with rStx2e (750 000CD50 per piglet) on day 40, and monitored for signs of ED until day 54. Sera from all piglets were tested for Stx2e neutralizing antibodies (Stx2e-nAb) with a Vero cell culture assay at days 9, 28, and 40 (before vaccination or challenge, respectively). Depending on the antigen dose, up to 100% of the piglets vaccinated with Stx2e toxoids survived the rStx2e challenge in contrast to placebo-vaccinated and non-vaccinated piglets (30.0% and 19.6% survivors, respectively). Survival rates were slightly higher in the rStx2e_662-group than in the rStx2e_F18_R167Q-group (94.7% versus 84.4%). Stx2e-nAb were only detectable in piglets vaccinated with Stx2e toxoids. Stx2e-nAb-titers were positively correlated with the administered rStx2e antigen dose. All piglets exhibiting a titer of ≥10 Stx2e-nAb on day 40 survived the subsequent rStx2e challenge while 78.0% of those with less Stx2e-nAb died or had to be sacrificed for ethical reasons.

Conclusions: We conclude that immunity against ED is critically based on antibodies capable to neutralize the Stx2e that has been resorbed from the intestine. The titer of Stx2e-nAb appears to be a valuable measure for the protective potential of Stx2e-based vaccines. Quantification of Stx2e-nAb may help to reduce the number of animals required for vaccines potency testing.
**P-131**

**Effects of Shiga Toxin 2 and Subtilase on Human Renal Tubular Epithelial Cells**

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**Introduction and Objectives:** Post-diarrhoea hemolytic uremic syndrome (HUS) is the most common cause of acute renal failure in children in Argentina. Renal damages have been strongly associated with Shiga toxin (Stx1) produced by *Escherichia coli* O157:H7 and other related strains. Those strains that express Stx2 are highly prevalent in Argentina. The Stx2 binds to the globotriaosylceramide (Gb3) on the plasma membrane of renal tubular epithelial cells. Several STEC non-O157 produce Subtilase (SubAB) that may contribute to HUS pathogenesis. The B subunits of SubAB bind to the surface of target cells via glycans displayed on glycoproteins. The purpose of the present work is to study and compare the cytotoxic effects of Stx2 and SubAB on primary cultures of human cortical renal tubular epithelial cells (HRTEC).

**Material and Methods:** HRTEC were isolated from kidneys removed from different pediatric patients undergoing nephrectomies at the Hospital Posadas, Buenos Aires, Argentina. The Ethics Committee of the Hospital approved the use of human renal tissues for research purposes. The cortex was dissected from the renal medulla and the primary cultures were performed according to the methods described previously (Silberstein et al., *Pediatr Nephrol* 23, 81, 2008). Cell viability was assayed by neutral red uptake in HRTEC incubated with different dilutions of Stx2 and SubAB, for 72 h. Apoptosis was analyzed at different times by acridine orange-ethidium bromide staining. Cell proliferation was measured at different times by incorporation of bromodeoxyuridine into the DNA of the cells, and detected using a specific antibody by fluorescence microscopy.

**Results:** The toxin concentration able to decrease HRTEC viability to 50% was 0.05 ng/mL for Stx2, and 5 ng/mL for SubAB. Incubation with Stx2 (10 ng/mL) for 6 h completely inhibited HRTEC proliferation, and produced 14 ± 3.1% apoptosis. After 24 h, Stx2 caused a significant increase in HRTEC apoptosis to 73 ± 15%. The pretreatment with C-9 (5 μM; Genzyme Corp.), a specific inhibitor of glucosylceramide synthase that inhibits the biosynthesis of Gb3, neutralized the cytotoxic effects of Stx2 on cell viability, cell proliferation, and apoptosis. Incubation with SubAB (40 ng/mL) did not alter the cell proliferation at 6 h. However, the incubation with SubAB for 24 h completely inhibited cell proliferation, and produced 28 ± 2.2% apoptosis. When cells were incubated with SubAB at 40 ng/mL, the results were similar to non-treated control cells.

**Conclusions:** SubAB may contribute to the tubular renal damage during the development of HUS. However, HRTEC are less sensitive to the cytotoxic effects caused by SubAB than those produced by Stx2.

**P-133**

**Escherichia coli** O104 Isolation Frequency and Immune Response Against O104 LPS in Dairy Cattle, from Different States of Mexico

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**Introduction and Objectives:** The ability of Shiga Toxin-Producing *Escherichia coli* (STEC) strains to cause severe disease is related to the production of Shiga toxins (Stx1, Stx2, and variants of Stx2), encoded on genome of lysogenic bacteriophages. The unprecedented outbreak of Shiga toxin/verotoxin-producing *E. coli* (STEC/VTEC) O104:H4 in Germany in May and June 2011, displayed several novel epidemiological, microbiological and clinical features about STEC strains. Due to the importance of meat products in food outbreaks, cattle have been the focus of many studies to determine its involvement in the transmission of the disease associated bacteria to humans. The aim of this study was the isolation and characterization of STEC strains from five herds localized in Central and Northwestern Mexico.

**Material and Methods:** Faecal and serum samples were obtained from 238 cattle, faecal samples were plated on MacConkey agar and five colonies of each culture were selected. The bacteria identification was performed with the Vitek automatized system and the serotyping was determined with 187 anti O and 56 anti H rabbit sera. The phylogenetic groups (A, B1, B2, and D) of the identified STEC strains, was determined by PCR assays with specific primers. Virulence genes stx1, stx2, ehxA and eae were analyzed by PCR assays. The bovine serum response against *E. coli* O157 and O104 LPSs and also against the *E. coli* K12, R1, R2, R3 and R4 cores was analyzed by ELISA assays.

**Results:** From the 238 faecal samples were isolated 656 colonies and identified as *E. coli* by the automatized Vitek system. The rabbit antiserum agglutination assay showed 204 different serotypes where STEC O26:NM, O104:H12, O111:H8, O116:H21, O145:NM, O146:H21, O157:H7, O176:H54, O179:H8, serotypes and non-STE O9 serogroup were identified. *E. coli* O104:H12 strains were isolated in 3 (7%) from one herd of 56 cows. The phylogenetic analysis showed that O104:H12 strains belonging to A commensal group. Virulence genes stx1, stx2, ehxA and eae were identified in the STEC strains. The analysis of bovine serum samples showed positive response against *E. coli* O157 (34%) and O104 (21%) LPSs and against K12 (36%), R1 (29%), R2 (21%), R3 (22%) and R4 (21%) cores.

**Conclusions:** Although *E. coli* O104 strains were not frequently isolated (3), a positive response of bovine serum samples (21%) against the O104 LPS was identified. Preliminary studies in the laboratory showed antigenic cross reactivity between *E. coli* O104 and O9:K9 LPSs, this observation suggest that the anti O104 LPS response of bovine serum could be related to intestinal bovine colonization by commensal enteric bacteria as *E. coli* O9:K9.

**P-134**

**Development of a Vitamin A-Deficient Mouse Model Susceptible to Shiga Toxin-Producing *Escherichia coli* Infection**

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**Introduction and Objectives:** *Shiga-toxin* (Stx)-producing *Escherichia coli* (STE) is a food-borne pathogen that can cause diarrhoea, haemorrhagic colitis and the life-threatening haemolytic uremic
syndrome (HUS), a systemic complication that affects 5–10% of infected children. Despite important knowledge obtained during last years, several components and mechanisms involved in HUS pathogenesis still remain unclear. An increasing number of reports highlight the critical role of vitamin A (VA) in the maintenance of the gut epithelium and in the homeostasis of the gut-associated lymphoid tissue (GALT). Considering this fact, the aim of this work was to develop a mouse model to study the influence of VA during STEC infection.

**Material and Methods:** To generate the VA deficiency, pregnant females were fed a VA-deficient diet since the second week of gestation and the litter received the same diet after weaning. Mice fed a VA-sufficient diet were used as control. Body weights were measured weekly and the level of retinol binding protein 4 (RBP4) in serum was measured monthly by ELISA as a surrogate marker of VA status.

**Results:** We determined that the level of RBP4 decreased progressively from week 8 to 12 in VA-deficient (VA-D) mice: for example, (mean µg/mL) 288.5 ± 67.9, STEC- mice: 57.8 ± 2.9, in infection.

**Conclusions:**

1. The percentage of monocytes-CR1+ was increased in both, classical and inflammatory subsets in HUS compared to HC (Results are expressed as the mean ± SD of the percentage: %CD16+CCR1+: HUS = 74.3 ± 7.8, HC = 47.6 ± 7.7, %CD16+CCR1+: HUS = 12.7 ± 4.5, HC = 4.2 ± 0.9, n = 8, *P < 0.05*). Moreover, the percentage of cells expressing CR5, a chemokine receptor that shares several ligands with CR1, was increased in both Monocyte subsets (*%CD16+CCR5*: HUS = 48.4 ± 13.2*, HC = 5.0 ± 1.5, *%CD16+CCR5*: HUS = 5.6 ± 1.7, HC = 2.3 ± 0.6, n = 3, *P < 0.05*).

2. Simultaneously, HUS patients showed an increased percentage of monocytes-CR2+ (*%CD16+CCR2+: HUS = 80.6 ± 4.0, HC = 82.5 ± 3.7, *%CD16+CCR2*: HUS = 14.8 ± 4.8*, HC = 4.1 ± 1.5, n = 8, *P < 0.05*).

**Poster Presentations**

P-136

**Altered Expression of Chemokine Receptors on Monocytes During Hemolytic Uremic Syndrome**

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**Introduction and Objectives:** Monocytes are central elements in the inflammatory response triggered during Hemolytic Uremic Syndrome (HUS). Different subsets of monocytes with distinct phenotypic and functional properties coexist in circulation. In healthy children (HC) around 80% of peripheral monocytes are CD14+CD16+, and are mainly characterized as CCR2+CCR5+CCR1+ cells (classical monocytes). Conversely the minor subpopulation, CD14+CD16+ cells (inflammatory monocytes), expresses CCR5 but reduced level of CCR2 and CCR1. Considering that chemokine receptors such as CCR1, CCR5 and CCR2 participate in different renal diseases, the aim of this work was to analyze whether the expression of these receptors is modulated in monocytes during HUS.

**Material and Methods:** For this purpose, the expression of CCR1, CCR2 or CCR5 in combination with CD16 was determined on monocytes considered as CD14+ cells, on whole blood from HUS patients in the acute period and HC by flow cytometry.

**Results:** HUS patients showed an expansion of inflammatory monocytes (%CD14+CD16+: HUS = 14.8 ± 3.5*, HC = 3.5 ± 0.5, n = 8, *P < 0.01*) in line with our previous results. We found that the percentage of monocytes-CR1+ was increased in both, classical and inflammatory subsets in HUS compared to HC (Results are expressed as the mean ± SD of the percentage: %CD16+CCR1+: HUS = 74.3 ± 7.7, HC = 47.6 ± 7.7, %CD16+CCR1+: HUS = 12.7 ± 4.5, HC = 4.2 ± 0.9, n = 8, *P < 0.05*). Moreover, the percentage of cells expressing CR5, a chemokine receptor that shares several ligands with CR1, was increased in both Monocyte subsets (%CD16+CCR5*: HUS = 48.4 ± 13.2*, HC = 5.0 ± 1.5, %CD16+CCR5*: HUS = 5.6 ± 1.7, HC = 2.3 ± 0.6, n = 3, *P < 0.05*). Simultaneously, HUS patients showed an increased percentage of monocytes-CR2+ (%CD16+CCR2+: HUS = 80.6 ± 4.0, HC = 82.5 ± 3.7, %CD16+CCR2*: HUS = 14.8 ± 4.8*, HC = 4.1 ± 1.5, n = 8, *P < 0.05*).

**Conclusions:** A common feature of our results is the increase in the frequency of monocytes expressing CCR1, CCR2 or CCR5 in HUS patients during the acute period. These alterations may be related to Monocyte activation or maturation as a result of the Shiga toxin-associated infection. Therefore, consider that the conventional Monocyte subset classification, according to the differential chemokine receptor expression, could be altered under inflammatory response developed in HUS. Moreover, it is known that in many infections and immune-mediated inflammatory responses, the recruitment of monocytes into inflamed tissues is mediated by CCR2 and CCR5 in response to their ligands: MCP-1, MIP1-α and RANTES. Interestingly, these chemokines are expressed by endothelial cells stimulated by inflammatory factors present during HUS. In this context, a greater acquisition of chemokine receptor could be involved in more capacity of recruitment or activation of monocytes, which finally contributes to the amplification of the renal inflammatory response.

P-138

**Factor VIII Administration in a Patient with Haemophilia A and STEC-Induced Haemolytic Uraemic Syndrome**

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**Introduction and Objectives:** The haemolytic uraemic syndrome (HUS) is a thrombotic microangiopathy leading to acute renal failure in children. In HUS patients it is triggered by STEC infection (typical HUS). Endothelial damage plays a central role in the pathogenesis of disease. Haemophilia A is a genetic disorder, leading to Factor VIII (FVIII) deficiency, an important factor in the coagulation system. Here we describe a haemophilia A patient that devel-
oped typical HUS. Increased amounts of FVIII had to be administered during the acute phase of the disease.

**Material and Methods:** At the age of 2 years and 9 months, our patient developed abdominal pain, vomiting, and bloody diarrhoea. He was diagnosed with typical HUS (STEC O26). On the second evening he developed a tonic-clonic epileptic insult. Bilateral symmetric signal intensity changes in the basal ganglia were seen on MRI. Because of further deteriorating renal function, symptoms of ileus, and severity of neurological condition, continuous venovenous haemofiltration was initiated as renal replacement therapy.

Due to hemorrhagic colitis, severe neurological complications, hypertension, and bleeding at the exit site of the jugular catheter, all in combination with thrombocytopenia and persistently low FVIII levels in serum, FVIII treatment had to be increased enormously.

**Results:** It is known that shiga toxin induces the secretion of von Willebrand Factor (vWF) from microvascular endothelial cells, a protein important in adhesion of platelets at the site of injury and a carrier of FVIII in plasma. The adhesion of platelets by vWF will probably lead to increased thrombus formation in the microvasculature of the kidneys of HUS patients. The supraphysiological dose of FVIII needed in this case may have contributed further to the thrombotic microangiopathy after shiga toxin-induced endothelial damage in the affected organs (especially in the intestines and the brain).

Renal function recovered after 18 days of renal replacement therapy, besides proteinuria and hypertension. After 3 months surgical intervention was needed for resection of colorectal stenosis and invagination. For his severe neurological involvement, an intensive rehabilitation program was necessary in which clear neurological progress was made, but the boy is still not fully rehabilitated.

**Conclusions:** To our knowledge, this is the first report of a haemophilia patient that developed HUS after STEC infection. The treatment of haemophilia in the acute phase of the HUS could have contributed to the difficult management of the disease and to the development of severe gastro-intestinal and neurological complications.

**P-141 Neurovascular and Behavioral Changes After Sub-Lethal Administration of Intravenous Stx2 and/or LPS in Mouse Brains**

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**Introduction and Objectives:** The CNS is usually affected in patients that suffer from hemorrhagic colitis and HUS by STEC. The aim of the present study was to determine the effects of Stx2 on the membrane bound complement regulators CD46, CD55 and CD59 in two different human renal cell lines, human kidney

**Material and Methods:** NIH mice (20–25 g) from both sexes were subjected to i.v. s-l administration of Stx2 (1/3500; DL₅₀ = 1/1500), or vehicle for morphologic and behavioral experiments. They were anesthetized, perfused and their brains processed for confocal (binding of biotinylated lectins to study the changes of the microvasculature profile (number and size of microvessels) and Fluorojade-B to determine neurodegeneration) or transmission electron microscopy (TEM). Another group of mice were subjected to the behavioral Shira (standard neurologic test), Object recognition (memory) and inclined plane (motor and balance) tests.

**Results:** Changes in body weight, blood urea and creatinine are observed following i.v. s-l Stx2 (P < 0.05). An increase in the microvasculature profile is determined after 2 days of iv s-l Stx2 administration, peaking at day 7 in hippocampus and striatum. In addition, the microvasculature profile produced by i.v. s-l Stx2+LPS is higher than Stx2, LPS or vehicle (P < 0.05). The Fluorojade-B histofluorescent technique shows neurodegeneration. TEM observation reveals among other features damaged neurons, astrocytes and oligodendrocytes with edema, shrunken endothelium and perivascular edema. Interrupted synopsis and mast cells extravasation are found. The Shira test shows mild neurological dysfunction after i.v. Stx2 at 2 days. Hind limb paralysis and lethargy are observed after 8 days. Also, the inclined plane test shows reduced motor ability and balance after 5 days (P < 0.05). Cognitive object recognition test shows significant differences found between 4 and 7 days. No behavioral and motor differences are found between treated and control groups after 20 days.

**Conclusions:** The i.v. s-l Stx2 is enough to change the microvasculature starting at 2 days. This correlates well with the cell damage revealed by Fluorojade-B and TEM, and with the observed behavioral dysfunctions (memory, motor and balance). LPS exacerbates the microvasculature profile done by Stx2. The microvasculature profile could be a suitable marker to study the neuropathogenic condition by STEC in animal models and in patients.

**P-142 Reduction of the Expression of Complement Regulator CD59 on Kidney Glomerular Endothelial Cells by Shiga Toxin 2**

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**Introduction and Objectives:** Enterohemorrhagic Escherichia coli (EHEC) virulence factor Shiga toxin 2 (Stx2) is the main cause for the hemolytic uremic syndrome (HUS) which is characterized by the clinical triad of hemolytic anemia, thrombocytopenia and acute renal failure. In addition to the EHEC-induced HUS there are inherited atypical forms of HUS (aHUS), caused by mutations in regulators of the complement system, such as the soluble factor H or the cell-surface associated CD46 (MCP). Mutations in other cell surface regulators, like CD55 (DAF) or CD59, have not been reported as cause of aHUS. Both CD46 and CD55 play a role in the deactivation of the C3- and C5 convertases, whereas CD59 modulates membrane attack assembly.

The aim of the present study was to determine the effects of Stx2 on the membrane bound complement regulators CD46, CD55 and CD59 in two different human renal cell lines, human kidney
glomerular endothelial cells (cGEnC) and human kidney proximal tubular epithelial cells (HK-2).

**Material and Methods:** Both cell lines were incubated with 20 pg/μL Stx2 at 37°C for 4 h and expression of CD46, CD55 and CD59 on the cell surface was assessed by flow cytometry.

**Results:** No influence of Stx2 on CD46, CD55 and CD 59 on HK-2 cells was found. On cGEnC cells a slight, but not significant decrease of CD46 and CD55 was detected. However a highly significant reduction (P < 0.01) of CD59 was shown for this cell line. ELISA of cell culture supernatant was negative for CD59, suggesting that the reduction of CD59 seen in flow cytometry is not caused by cleavage from the cell membrane.

**Conclusions:** Together with our previous findings, showing that Stx2 activates the complement system, we draw the conclusion that Stx2 additionally reduces the complement regulator CD59 on the cell surface of human kidney glomerular endothelial cells which implies that the cells become more vulnerable against complement attack.

**P-143**

**Colonisation and Shiga Toxin Translocation of Enterohaemorrhagic E. coli in a Microaerobic Human in vitro Infection Model**

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**Introduction and Objectives:** Advances in the understanding of the pathogenesis of enterohaemorrhagic E. coli (EHEC) have greatly benefited from the use of human epithelial cell lines under aerobic (AE) conditions. However, in the target site of EHEC infection, the human intestine, conditions are microaerobic (MA). The aims of our study were to investigate the influence of reduced oxygen levels on EHEC colonisation, Shiga toxin (Stx) production and translocation.

**Material and Methods:** We have developed and applied a vertical diffusion chamber system using polarised T84 human colon carcinoma cells. Cells were infected with EHEC O157:H7 EDL933 or inoculated with purified Stx. Incubations were carried out under apical AE or MA conditions.

**Results:** While apical microaerobiosis and EHEC infection did not affect cell integrity and barrier function, numbers of adherent bacteria were significantly increased under MA compared with AE conditions. In addition, expression and translocation of EHEC type III secreted (T3S) effector proteins was considerably enhanced by the MA environment and dependent on the presence of host cells. Increased colonisation was mainly mediated via formation of EspA filaments as adherence levels of an isogenic deletion mutant were not influenced by low oxygen levels. In contrast to colonisation and T3S, Stx production and release into the apical medium was significantly reduced under MA compared with AE conditions. However, Stx translocation across the polarised cell monolayer was enhanced under microaerobiosis resulting in elevated Stx levels in the basal compartment. Increased MA Stx transport was dependent on EHEC infection and was not observed with purified Stx. Epithelial barrier function was maintained during infection indicating a transcellular pathway of toxin translocation.

**Conclusions:** Taken together, this study indicates that EHEC colonisation, Stx production and translocation are likely to be influenced by the MA environment in the human gut and therefore underlying mechanisms might be different than in conventional AE cell culture systems.

**P-144**

**Subcellular Distribution of Shiga Toxin Receptors and Expression Profiles of Related Glycosyltransferases in Lymphoid and Myeloid Cell Lines**

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**Introduction and Objectives:** Some strains of human pathogenic Escherichia coli, such as E. coli serotype O157:H7 and the recent 2011 German outbreak strain E. coli O104:H4, produce Shiga toxins (Stxs), the major virulence factors involved in potentially lethal complications. Stxs belong to the group of ribosome-inactivating proteins and are composed of an enzymatically active A-subunit and five identical B-subunits. The A-subunit of Stxs has RNA N-glycosidase activity and inhibits eukaryotic protein biosynthesis. The B-pentamer is responsible for attachment to the high and less effective glycosphingolipid (GLS) receptors globotriaosylceramide (Gb3Cer/C D77) and globotetraosylceramide (Gb4Cer), respectively. In our former studies Gb3Cer (d18:1, C24:1/C24:0) and Gb3Cer (d18:1, C16:0) were identified as the prevalent Stx-receptors in lymphoid (B-cell derived) and myeloid (monocytic) cell lines. Gb4Cer (d18:1, C24:1/C24:0) and Gb4Cer (d18:1, C16:0) were additionally characterized in a myeloid cell line. It is believed that organization of GLS receptors in lipid raft microdomains is the prerequisite for effective binding and internalization of Stxs. In this study we characterized in detail the microdomain-association of GLSs in lymphoid and myeloid cell lines using detergent resistant membranes (DRMs) obtained by sucrose density gradient ultracentrifugation.

**Results:** Here we clearly show the association of Gb3Cer and Gb4Cer with DRM microdomains and characterize the membrane environment within the different membrane fractions of these cell lines. Moreover, studies on the expression of Lc2Cer, Gb3Cer and Gb4Cer syntheses by means of real time PCR showed high compliance with solid phase immunodetection assays and Stx-mediated cytotoxicity.

**Conclusions:** Our comprehensive expression analysis of Stx-receptors and the determination of their structural diversity as well as subcellular distribution in lymphoid and myeloid cell lines provide the basis for further exploring the functional role of lipid raft-associated Stx-receptors in cells of leukocyte origin. This work is supported by grants from the Deutsche Forschungsgemeinschaft (DFG)-funded International Graduate School (GRK 1409).

**P-145**

**Involvement of Intimin, Tir and Type Three Secretion System of an Atypical Enteropathogenic Escherichia coli Strain in Invasion of Caco-2 Cells**

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**Introduction and Objectives:** Enteropathogenic Escherichia coli (EPEC) are classified as typical (tEPEC) and atypical EPEC (aEPEC). tEPEC express the Bundle-forming pilus while aEPEC lacks these fimbria. Both groups produce attaching and effacing (A/E) lesions that are characterized by an intimate bacterial adherence to enterocytes mediated by the adhesin intimin and its receptor (Tir). Tir is a bacterial effector, which is translocated into the enterocytes by a Type Three Secretion System (T3SS). Previously, we showed that aEPEC strain 1551-2 invades HeLa cells in an intimin-dependent manner.
mechanism since an isogenic Intimin mutant was non-invasive. Subsequently, we demonstrated that aEPEC 1551-2 also invades intestinal T84 cells, which represent enterocytes of the colon. In this work, we evaluated aEPEC 1551-2 regarding its ability to adhere and to invade differentiated Caco-2 cells (representing small intestine enterocytes) and the participation of intimin, Tir and T3SS in these processes.

**Material and Methods:** Isogenic T3SS and Tir mutants were constructed by insertion mutagenesis in the escN gene and by deletion of the tir gene, respectively. The intimin-deficient mutant was constructed previously. Invasiveness was evaluated after infection with 10^7 bacteria/well for 6 h, following the gentamicin-protection assay (100 µg/mL) and lysis. Invasion indexes were calculated as the percentage of the total number of cell associated bacteria that were in the intracellular compartment.

**Results:** aEPEC 1551-2 invaded Caco-2 cells fourfolds more efficiently than tEPEC strain E2348/69 (5.818 ± 1.327 and 0.7096 ± 0.4447, respectively). Adherence of the intimin and Tir mutants was preserved, but their invasive ability was significantly reduced (from 5.818 ± 1.327 to 0.17 ± 0.08 and 0.07086 ± 0.06108, respectively). In contrast, the T3SS mutant was 55% less adherent than the wild type strain and non-invasive, suggesting that aEPEC 1551-2 has no alternative adhesion and/or intimin-receptor that could trigger Caco-2 invasiveness. To confirm this hypothesis, a complemented T3SS mutant strain was obtained and its interaction with Caco-2 cells showed an invasion index similar to that of the wild type strain.

**Conclusions:** Altogether our data suggest that aEPEC 1551-2 may adhere to and invade enterocytes of both large and small intestines. Invasion of Caco-2 cells depends on intimin-Tir interaction since both mutants prevented this interaction and were non-invasive.

**P-147**

**Investigation of Different Modes of Action of Shiga Toxin 1 and 2**

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**Introduction and Objectives:** The ability of enterohemorrhagic E. coli to cause hemorrhagic uremic syndrome (HUS) depends on the type of Shiga toxin (Stx) which they produce. In particular, infections with EHEC producing Stx2 as the only toxin significantly more frequently progress to HUS than infections with EHEC producing Stx1 only or both toxins together. Endothelial cells are the major toxin targets involved in the pathogenesis of HUS. Therefore, we performed a direct, comprehensive, and systematic comparison of cellular injuries induced by Stx1 and Stx2 on microvascular and macrovascular endothelial cells.

**Material and Methods:** Human brain microvascular endothelial cells (HBMECs) and macrovascular human umbilical vein endothelial cell (HUVEC)-derived EA.hy 926 cells were infected with different doses of purified Stx1 or Stx2. Stx-mediated effects were analysed using classical methods (protein inhibition and cytotoxicity assays), scanning electron microscopy of microcarrier-grown endothelial monolayers, and visualization of living cells using digital holographic microscopy. The mode of cells death was verified using DNA fragmentation assays and flow cytometry.

**Results:** Although no differences could be observed between Stx1 and Stx2 regarding the inhibition of protein biosynthesis, we found different mechanisms of Stx1 and Stx2 toxicities. Stx1 induces both apoptosis and necrosis, while Stx2 induces almost exclusively apoptotic mechanisms in both endothelial cell lines investigated. In addition, microvascular and macrovascular endothelial cells have different susceptibilities to the toxins: EA.hy 926 cells are slightly, but significantly (~10 times) more susceptible to Stx1, whereas HBMECs are strikingly (~1000 times) more susceptible to Stx2. Thus, the macrovascular endothelial cells are relatively resistant, whereas microvascular cells are extremely susceptible to Stx2.

**Conclusions:** Our findings have implications in the pathogenesis of HUS. They suggest that Stx2 is the predominant Stx type involved in the microvascular endothelial damage underlying this disorder, which is in accordance with epidemiologically determined association between Stx2 production by the infecting EHEC and HUS development. Moreover, the existence of yet to be delineated Stx type-specific mechanism(s) of endothelial injury needs to be further investigated.

**P-148**

**Verotoxin (VT) of Enterohaemorrhagic Escherichia coli (EHEC) Promotes Bovine Intestinal Colonisation**

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**Introduction and Objectives:** Enterohaemorrhagic Escherichia coli (EHEC), particularly of serotype O157:H7, are the most common cause of haemorrhagic colitis (HC) which can lead to life-threatening haemolytic uraemic syndrome (HUS) in humans. Verotoxins (VT; or shiga-like toxins) are major virulence factors and are key determinants in the pathophysiology of EHEC infections in humans. Cattle are an important reservoir host for EHEC in which infection occurs asymptomatically. Consequently the significance of virulence determinants, including VT, in the bovine host is obscure.

**Materials and Methods:** Initial work identified potential contribution of VTs to bacterium-host interactions and this investigation aimed to characterise roles of VT in colonization of bovine intestinal epithelium by EHEC. For this, adherence of a panel of wild-type and mutant (VT-negative) EHEC strains to primary epithelial cells from the most terminal part of rectum (the principal site of EHEC colonization in cattle) was conducted (tissue obtained from the abattoir).

**Results:** Carriage of VT was associated with greater adherence to epithelium as demonstrated by higher capacity to form micro-colonies. Pre-treatment of cells with VT produced a similar phenotype. VT enhanced expression of eukaryotic protein Annexin II which co-localised with adherent bacteria forming attaching and effacing lesions on the bovine epithelium indicating its role in O157 colonization. Further work has shown that Annexin II binds to one of the EHEC outer membrane proteins suggesting its role as a potential O157 receptor at the bovine terminal rectal epithelium.

**Conclusions:** VT of EHEC therefore do not show classic cytotoxicity for bovine intestinal epithelium but do exert pleiotropic effects on these cells, by modifying epithelial surface hence enabling EHEC colonization.

**P-231**

**Inhibitory Effect of Probiotic E. coli Nissle 1917 on Growth, Shiga Toxin Release and Adhesion of Major EHEC Serotypes**

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**Introduction and Objectives:** As there is no consensus regarding the use of antibiotics for the treatment of Enterohemorrhagic E. coli...
(EHEC) mediated illnesses, alternatives like probiotics are of increasing interest. In this study, we analysed the effect of the well known probiotic *E. coli* strain Nissle 1917 (EcN) on growth viability, Shiga toxin release and adhesion of strains of EHEC serotypes O26:H11, O103:H2, O111:NM, O145:NM and O104:H4.

**Materials and Methods:** EHEC strains were co-cultured with EcN (equal ratio) in LB broth and reduction in growth viability of EHEC strains was measured as CFU count on LB agar plates containing streptomycin. Stx-ELISA determined the difference in Shiga toxin release from co-culture broths (EcN + EHEC) relative to toxin release from controls. In addition, *in vitro* probiotic effects of pre-incubated EcN on EHEC adhesion efficiency were evaluated on human colonic epithelial cells (Caco-2). In all experiments, *E. coli* MG1655 served as control.

**Results:** In co-culture experiments, EcN caused considerable reduction in growth and Shiga toxin release (20–99%) depending on the serotype of the EHEC strain. This effect was not caused by EcN-microcin. Furthermore, pre-incubation with EcN resulted in marked decrease (78–99%) of adhesion efficiency of EHEC strains whereas non-probiotic *E. coli* MG1655 had no inhibitory effect.

**Conclusions:** These results show that probiotic EcN displays strong inhibitory effects on growth, Shiga toxin release and adhesion of major EHEC serotypes. Thus, EcN is a putative therapeutic candidate during EHEC-mediated diseases.
Animal Reservoir, Food, Environmental Contamination and Transmission

Prevalence and Characterization of Shigatoxin-Producing *Escherichia coli* Isolated from Raw Meats, Ready-to-Eat Meat Products, Water and Human Diarrhoeal Samples in Punjab, India

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**Introduction and Objectives:** The present study was undertaken to assess the prevalence of shigatoxin producing *Escherichia coli* (STEC) prevalence in raw meats, ready-to-eat (RTE) meat products from retail markets, drinking water and human diarrhoeal samples in Punjab. Another objective was to phenotypically and genotypically characterize the STEC isolates for their virulence and antibiotic resistance.

**Material and Methods:** A total of 675 samples from various sources comprising, 392 raw meats (pork-106, buffalo meat-69, chevon-68, mutton-55, fish-54, chicken-40), 167 RTE meat products (buffalo meat-40, chicken-40, pork-25, fish-27, mutton-25, chevon-10), 64 water and 52 human diarrhoeal samples collected from hospitalized patients were subjected isolation and direct multiplex PCR to detect virulence genes (stx1, stx2, eaeA, hlyA). *E. coli* isolates were sero-grouped on the basis of their ‘O’ antigen. Vero cell cytoxicity assay, Congo red binding assay and hemolysis production on 5% sheep blood agar were carried out. Antibiotic susceptibility was tested by disk diffusion using 20 antibacterials: amoxicillin (30 μg), amoxicillin (30 μg), ampicillin (10 μg), cefadroxil (30 μg), cefotaxime (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), colistin (10 μg), co-trimoxazole (1.25/23.75 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), linezolid (30 μg), norfloxacin (10 μg), ofloxacin (5 μg), penicillin G (10 μg), polymyxin B (300 U), streptomycin (10 μg), tetracycline (30 μg) and trimethoprim (5 μg). STEC isolates resistant to tetracycline and streptomycin were subjected to PCR for detection of tetA, tetB, tetC, strA and strB genes.

**Results:** Out of 675 samples subjected to PCR, 203 samples (30% were positive for STEC (41% of raw meats, 11% of RTE meat products, 28% of water and 13% human diarrhoeal samples). All STEC isolates carried stx1, 65%, 44% and 66% STEC isolates harboured stx2, eaeA and hlyA genes, respectively. Phenotypically, 99% of STEC were verocytotoxigenic. 84% exhibited Congo red binding and 42% were hemolytic on sheep blood agar. Serogroups (total 16; O: 4, 7, 8, 9, 15, 26, 22, 91, 103, 105, 110, 113, 126, 128, 153, 168) that originated from human samples were also isolated from food samples. Seventy-two STEC isolates were resistant to more than 10 (50%) antibiotics tested. Genotypically the most common tetracycline resistance genes were tetA (60%) and tetB (27%) while 12% of STEC isolates possessed both genes. Only one isolate from chicken sausage harboured tetC. For strA and strB genes, 47% of STEC isolates harbored both genes, 33% carried strA and 10% possessed strB, while 10% of isolates did not carry any.

**Conclusions:** The prevalence of multidrug resistant STEC in raw meats, RTE meat products, and water samples was high which is of great concern to food safety and public health.

Shiga Toxin-Producing *Escherichia coli* O157:H7 Isolated from Cattle Feedlot Effluents

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**Introduction and Objectives:** Major outbreaks due to the consumption of raw fruits and vegetables or accidental ingestion of water contaminated by Shiga toxin-producing *Escherichia coli* (STEC) have been increasingly reported. Fresh produce may have been contaminated by direct spreading of cattle manure on growing crops or indirectly via contaminated irrigation. STEC O157:H7 has been shown to survive for long periods in water-trough sediments, and troughs can serve as a reservoir for infection. The research was intended to study the prevalence of STEC O157:H7 in water samples of cattle feedlot effluents in Entre Ríos Province, Argentina.

**Material and Methods:** Between April 2009 and July 2011, 320 water samples of cattle feedlot effluents in 10 farmers were studied. Two-liter samples were kept for 24 h at room temperature in bottles with Moore swabs. Swabs were incubated in trycicase soy broth (TSB) for 5 h, and then exposed to an acid shock (pH 4.0) for 30 min before neutralization with TSB-Tris (pH 8.7) and incubation at 42°C for 24 h. Immunomagnetic separation for *E. coli* O157 was performed and the immunoenconcentrate was streaked onto sorbitol MacConkey agar, O157:H7 ID3™ and Chromagar™ O157. After incubation at 37°C for 24 h, the confluent growth zone and individual colonies were screened for stx1, stx2 and rfbO157 genes by a multiplex PCR. STEC isolates were characterized by biochemical tests, serotyping, stx-genotyping, and by pulsed-field gel electrophoresis (PFGE) using the 24-h PulseNet standardized protocol.

**Results:** By PCR, 43 (13.4%) water samples were rfbO157-stx-positive and 38 (11.9%) STEC O157 strains were isolated. In addition, 172 (53.8%) samples were rfbO157-positive and 76 *E. coli* O157 strains were recovered. STEC O157 strains were recovered from wastewater feedlot (19/129, 14.7%), waste stabilization ponds (11/130, 8.5%), and watercourses or lagoons near feedlot (8/61, 13.1%). The most prevalent stx-genotype was stx1/stx2a (50%) followed by stx1 (47.4%). All STEC strains harbored the eae, ehxA, and flcH genes. The clonal relatedness of 38 STEC O157 strains was established by XbaI-PFGE. The analysis showed 16 different patterns with 83.9% similarity, with 29 strains grouped in seven clusters (I to VII) of 2–10 strains each one and 100% homology. Unique patterns were observed for nine strains. Some XbaI-PFGE patterns identified are included in the Argentine Database of *E. coli* O157, corresponding to strains isolated from HUS and diarrhea cases, food, and animals.
Among them, AREXHX01.0022 is the second prevalent pattern, representing 5.5% of the total.

**Conclusions:** Control strategies must be considered on cattle farms, in order to limit entry of STEC into the environment. As O157 is not a highly competitive microorganism in aquatic environments, waste stabilization ponds would be useful for minimizing its spreading, but this strategy is insufficient.

**P-153**

**Characterization of New Emerging Serogroup O178 Shiga Toxin-Producing *Escherichia coli* Strains**

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**Introduction and Objectives:** Shiga toxin-producing *Escherichia coli* (STEC) is a zoonotic pathogen of significant public health concern and more than 400 O:H types of STEC are associated with infections in humans. Over the last years, the *E. coli* serotyping scheme has been extended by the newly defined serogroups O174 to O186. Because only a few reference laboratories perform complete *E. coli* serotyping, little is known about the epidemiology and human pathogenicity of these new *E. coli* types. As STEC O178 strains have been frequently isolated from cattle and food in South-America and Europe, we became interested in their virulence characteristics. We have analyzed a collection of 69 *E. coli* O178 strains for their virulence properties and investigated the genetic relationships among STEC O178 by pulsed-field gel electrophoresis (PFGE).

**Material and Methods:** Sixty-nine *E. coli* O178 strains from animals, food and humans from Argentina and Germany were investigated. Sixty-one of these were STEC O178:H19, six were O178:H7 and one each O178:H10 and O178:H16. The production of Shiga toxins (Stx) was tested by the Vero cell assay and a Stx ELISA. Subtyping of Stx1 and Stx2 encoding genes was performed by PCR and RFLP analysis of PCR products. The presence of accessory virulence genes (eae, ehxA, espP, katP, etpD, subA, saa, iha, ent, nleB, nleE, nleF, nleH1-2, nleA) was determined by a low-density macroarray. PFGE with XbaI digested total DNA of bacteria was performed using the CDC PulsNet protocol.

**Results:** Production of Stx and stx-genes were found in all O178:H19, four O178:H7 and the O178:H10 strain. Except for one O178:H7 EPEC strain all others were negative for eae, katP, etpD and nle genes. Subtyping of the 69 strains by PFGE revealed 52 patterns. Fifty-six of the 61 O178:H19 strains grouped into two major clusters. Cluster 1 is formed by 31 strains (>83% similarity) characterized by HEC virulence plasmid genes (ehxA and saa) and stx1a (*n* = 26), stx2a (*n* = 30) and/or stx2d-activatable (*n* = 5) genes. Cluster 2 (>87% similarity) is formed by 25 strains with stx2v-ha (*n* = 21) or stx2v-hb (*n* = 4) genes but negative for the HEC-virulence plasmid genes. Both O178:H19 clusters contain strains of different source and geographical origin showing similarities up to 100%. Five O178:H19 as well as all non-H19 O178 strains were genetically more distant (59-72% similarity). Stx1c (*n* = 4) and Stx2b (*n* = 3) were associated with O178:H7 strains from humans, animals and deer meat.

**Conclusions:** According to their virulence genes most of the STEC O178 strains are potentially able to cause disease in humans. Among the human O178 isolates, one O178:H19 strain (cluster 1) isolated from a HUS patient had the same PFGE genotype as a bovine isolate. The other human strains were O178:H7 from diarrhoeic patients. STEC O178 is possibly underdiagnosed in humans as most diagnostic laboratories do not have the complete serotyping facilities.

**P-154**

**Application of a Real-Time PCR-Based Approach for Monitoring of O26, O103, O111, O145, and O157 Shiga toxin-Producing *Escherichia coli* in Cattle at Slaughter**

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**Introduction and Objectives:** Most cases of severe STEC infections in humans are attributed to STEC O157:H7, but the importance of non-O157 STEC is increasingly recognized. For monitoring of STEC in cattle, the European Food Safety Authority (EFSA) proposed to screen for STEC O26, O103, O111, O145, and O157, the so-called top-five serogroups. The aims of this study were (i) to screen bovine samples by real-time PCR for Shiga toxin genes and if positive for the top-five serogroups, and (ii) to isolate strains from a collective of O26, O145, and O157 positive samples. Thus, application and challenges of this monitoring approach in cattle were evaluated.

**Material and Methods:** From 572 slaughtered cattle in the age from 3 to 24 months, fecal samples were collected in seven Swiss abattoirs. After enrichment (nTSB with novobiocin), samples were screened by real-time PCR first for stx and then (if positive) for the top-five STEC serogroups O26 (wzx), O103 (wzx), O111 (wddl), O145 (iibp1), and O157 (rfeE). From a selected collective of O26, O145, and O157 positive samples, isolation of O26, O145, or O157 strains was attempted by colony dot-blot hybridization after immunomagnetic separation. Isolated strains were then examined for the presence of stx1, stx2, and eae (intimin).

**Results:** Of 562 samples with evaluable results, 74.2% tested positive for stx. Amongst them, serogroups O145, O103, O26, O157, and O111 were detected in 41.9%, 25.9%, 23.9%, 7.8%, and 0.8%, respectively. With regard to the 363 samples analyzable with all serogroup-specific assays, 132 (36.4%) tested negative for all five serogroups. One, 2, 3, and 4 of the top-five serogroups were detected in 136 (37.5%), 67 (18.5%), 22 (6.1%), and 6 (1.7%) samples, respectively. From 91 O26, 158 O145, and 28 O157-positive samples, only 16 O26, 27 O145, and ten O157 strains were isolated. The O26 strains were all eae-positive, but only eight harbored stx (stx1). Of the 27 O145 strains, 10 were eae-positive including four harboring stx1 or stx2, whereas 17 were negative for stx1, stx2, and eae. Furthermore, five of the 10 O157 strains harbored stx2 and eae and were identified as STEC O157:H7:H-. The other five O157 strains were negative for stx and eae or positive only for eae.

**Conclusions:** Shiga toxin genes were frequently found in fecal samples from young Swiss cattle at slaughter and the top-five STEC serogroups, especially O26, O103, and O145, were also detected amongst the stx-positive samples. But such screening does not result in a bacterial isolate and isolation of respective strains poses a major challenge. Success rates in isolation were low and only few of the isolated strains showed a virulence pattern of human pathogenic STEC. Thus, the value of a STEC monitoring with the described approach remains questionable.
P-156
Seasonal Prevalence of Escherichia coli O157:H7 and Generic E. coli on Hides of Cattle at Slaughter
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Introduction & Objectives: Escherichia coli O157:H7 has proven difficult to control due to erratic fecal shedding by cattle and the low dose which can result in human disease. A study was conducted at a beef abattoir in southern Alberta, Canada to identify and quantify hide swabs positive for generic E. coli and E. coli O157:H7.

Material & Methods: Eighteen hide swabs were collected at approximately hourly intervals during two shifts per day for 5 days per week, over a period of 8 weeks in January, February and March 2010 and 8 weeks in June and July 2010. Swabs were hydrated by addition of 8 mL of 0.1% (v/v) peptone water supplemented with 10% (v/v) glycerol. The hide was swabbed with a sponge in an area of 500 cm² behind the foreleg of each carcass immediately prior to slaughter. The hide was then cut into pieces and frozen in liquid nitrogen. Each hide was then thawed for analyses of E. coli O157 and generic E. coli, with E. coli O157:H7 confirmed by multiplex PCR analyses of stx1, stx2, intimin (eae) and flagellar H7 (flgC) genes.

Results: Of 1277 swabs, 71 were positive for E. coli O157:H7 by immunomagnetic separation, but numbers of E. coli O157:H7 on hides were below limits of enumeration (<40 CFU/cm²). Detection of E. coli O157:H7 was highest (P < 0.05) in March and July, while generic E. coli were higher (P < 0.05) in summer than winter months and varied by day of the week (P < 0.05) according to slaughter plant hygiene schedules. Peak detection of E. coli O157:H7 in March was unexpected and may have been influenced by daily freeze-thaw fluctuations in ambient temperature (>15°C) leading to increased stress of cattle. Carcasses with highest levels of generic E. coli (>4.5 log CFU/500 cm²) had a higher frequency of detection of E. coli O157:H7 (P < 0.001), although for the majority (82.4%) of carcasses there was no relationship between level of generic E. coli and incidence of E. coli O157:H7.

Conclusions: Factors other than carcass hygiene may impact incidence of E. coli O157:H7 on hides at slaughter. During months when detection of E. coli O157:H7 was highest (March and July), daily ambient temperature also varied by at least 15°C for the majority of days per month. Consequently, it is possible that wide ranges in ambient temperature prior to slaughter may be an additional risk factor for shedding of E. coli O157:H7 by cattle, although controlled studies to evaluate impacts of climate would be challenging due to the erratic nature of shedding of this organism. Pre-intervention hides that were heavily contaminated with generic E. coli were at a greater risk for contamination with E. coli O157:H7, but numbers of E. coli O157:H7 at the site evaluated were so low as to make it unlikely that any would survive subsequent carcass decontamination.

P-157
Virulence of Extended-Spectrum ß-Lactamase-producing Escherichia coli in cattle
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Introduction & Objectives: Cattle are known reservoirs of virulent Escherichia coli serotypes frequently incriminated in human infections associated with Shiga-toxin producing (STEC) and enterohemorrhagic (EHEC) strains. In line with the possible emergence of Extended-Spectrum Beta-Lactamases (ESBLs) in E. coli isolates of highly virulent serotypes, such as the O157:H7, O26:H11, O111:H8 or more recently O104:H4 serotypes, we investigated the proportion of virulent serotypes and virulence factors in a collection of 204 ESBL-producing E. coli isolates from faecal content of diarrheic cattle, recovered from 2006 to 2010 through the National Network for the Surveillance of Resistance to Antimicrobials in Animals in France.

Material & Methods: After phylogenetic grouping and identification of the ESBL genes, the strains were screened using specific primers for the detection of the stx1, stx2, eae and espP virulence genes. The serotyping of positive strains and the characterization of virulence determinants was performed by commercial DNA array (Alere France). In addition, subtypes of eae (β1, β2 and β3) and genomic O island 122 (O1-122) were identified.

Results: ESBL genes were mostly of the CTX-M-1 (65.7%) and CTX-M-9 (27.0%) groups. Only 3.9% of the E. coli isolates carried the blaCTX-M-2 gene whereas the remaining ESBL-positive strains (3.4%) harbored non blaCTX-M genes. The CTX-M-1-producing strains were significantly more of phylogroup A than of phylogroups B1 (P = 0.0001) and D (P = 0.0008). Fifty-seven of the 204 strains (27.9%) carried the espP virulence gene. The number of espP-positive strains was significantly higher in phylogroup D than in group A (P = 0.003) and our results approached significance (P = 0.053) when comparing D with B1. The number of detected virulence factors (VF) was significantly higher in phylogroup B1 than in phylogroups A (P = 0.04) and D (P = 0.02). No clustering of the CTX-M groups according to the VFs carriage was identified. Finally, two EHEC serotypes were identified in this collection, E. coli O111:H8 carrying the blaCTX-M-15 gene and E. coli O26:H11 carrying the blaCTX-M-9 gene. Both harbored stx1, eaeβ1 and espP-positive and belonged to biotype B1.

P-159
Isolation, Molecular Characterization and Antibiogram of Shiga Toxin-Producing Escherichia coli from Raw Chicken Meat in Tirupati, Andhra Pradesh, India
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Introduction & Objectives: The study was designed to ascertain the prevalence of shiga toxin-producing Escherichia coli from raw chicken meat collected from various retail meat shops in Tirupati, Andhra Pradesh, India, their molecular characterization to detect the virulence genes and to determine their antibiotic pattern so as to identify the best therapeutic regime and to control the spread of antibiotic resistant strains.

Material & Methods: A total of 68 raw chicken meat samples were collected from various retail meat shops and Escherichia coli was isolated from the samples and they were subjected for direct multiplex PCR to detect the virulence genes like stx1, stx2, eaeA and hlyA. Sero grouping of isolated Escherichia coli was carried out based on their O antigens. Blood agar plate test was done to find out the haemolysis production by the isolates. The antibiogram patterns of isolated strains were determined on Muller–Hinton agar by using disc diffusion method and in this test 20 antibiotic discs were used viz., tetracycline (30 µg), polymyxin B (300 units), chloramphenicol (30 µg), cephalothin (30 µg), kanamycin (30 µg), trimethoprim/Sulphamethoxazole (25 µg), ampicillin (20 µg), nitrofurantoin (300 µg), streptomycin (10 µg), amikacin (30 µg), gentamycin (30 µg) and trimethoprim (30 µg).
(10 μg), neomycin (30 μg), ofloxacin (5 μg), piperacillin (100 μg), cefotaxime (30 μg), cefuroxime (30 μg), cefazolin (30 μg), cefapime (10 μg) and lomefloxacin (10 μg).

**Results:** A total of 68 *Escherichia coli* were isolated from the raw chicken meat samples collected from various retail meat shops. All the isolates were subjected to multiplex PCR by using touch down protocol. Out of 68 isolates 21 (30.88%) samples were positive for shiga toxin–producing *Escherichia coli*. Among these 21 shigatoxin – producing *Escherichia coli*, six (28.57%) isolates carried stx1, stx2, eaeA and hlyA genes; two (9.52%) isolates carried stx1, one (4.76%) isolate carried stx1, six (28.57%) isolates carried stx1 and eaeA, three (14.28%) isolates carried hlyA and another three (14.28%) isolates carried stx1, stx2, and hlyA genes. Phenotypically 29 (42.64%) isolates were haemolytic on sheep blood agar. Among the isolates, 18 were typed as O serogroup and they were O: 18, 20, 21, 171, 109, 11, 172, 128, 29, 4, 7, 8, 9, 15, 26, 22, 110 and 153. The antibiogram of the isolates revealed that 36 shiga toxin–producing *Escherichia coli* were resistant to 15 antibiotics tested.

**Conclusions:** From this study it was observed that there is a prevalence of multi drug resistant shiga toxin–producing *Escherichia coli* in raw chicken meat available in retail meat shops of Tirupati, Andhra Pradesh, India which is having greatest public health significance and threat to the human health.

**P-160**

**Phages Cocktail Challenge Test for Biocontrol and Determination of Bacteriophage Inensitive Mutants on Non-O157 and O157:H7 VTEC**

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**Introduction & Objectives:** Verotoxigenic *Escherichia coli* strains (VTEC) biocontrol, mediated by phages, may be an interesting tool to control enteric diseases. However, bacteriophage insensitive mutants (BIMs) are naturally found in bacterial populations. Challenge test was performed to evaluate cocktails and individual phages biocontrol on non-O157 VTEC and O157:H7 VTEC strains (an O18 food isolate stx1+, stx2+ and a clinical isolate eaeA+, stx2+).

**Material & Methods:** BIMs were isolated by extended challenge test (48 h) -secondary culture, SC- of bacteriophage/sensitive E. coli strain systems and confirmed by the liquid culture sensitivity test. In addition, emergence frequency, reversion and stability were tested for each confirmed-BIM, through successive subcultures and infections. Ten stock phages (LM1-4 and DT1-6) were isolated, by the technique of double-layer agar (soft Hershey supplemented with MgSO4), from feces of healthy patients of the Centenary Hospital in Rosario city, Argentina.

**Results:** When testing bacteria versus phages, greater reductions were observed after 2 h a 37°C in non-O157 VTEC/DT5, DT6 and cocktail challenge test, being 6.38 log -cocktail- the maximum reduction achieved. On the other hand, for O157:H7 VTEC the greatest reduction was 5.23 log -cocktail- after 6 h of incubation. After 6 and 24 h, all reductions were significant for both strains tested, except for O157:H7 VTEC/DT1 system after 24 h. For all bacteriophage/E. coli sensitive strain systems, a significant bacterial growth was observed at 37°C after 48 h (SC), suggesting BIMs production under these tests conditions. Presumptive BIMs, isolated by SC, showed a 75% of confirmed BIMs and their frequencies, for both evaluated strains, ranged between 3.7 x 107 and 1.8 x 108. Only one BIM showed reversion to phage (DT6) sensitivity, while BIMs evaluated for phage DT1 were resistant. In stability trials, mutants isolated from non-O157 VTEC maintained the resistant phenotype until the second and third -with DT5- and sixth -with DT6- subculture, while BIMs isolated from O157:H7 VTEC up to the fourth -DT6- and only one until seventh -DT1- subculture.

**Conclusions:** We conclude that phages are very valuable to eliminate or significantly reduce the population of non-O157 VTEC and O157:H7 VTEC in vitro and the cocktails (DT1 + DT6 and DT5 + DT6) showed an additive effect. *Escherichia coli* confirmed-BIMs showed variable reversion and stability and a very low emergence frequency, being no obstacle in using bacteriophages as biocontrol tools against VTEC.

**P-164**

**Prevalence, Serotypes and Virulence Profile of VTEC Isolates from Healthy Slaughtered Sheep in Sardinia**

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**Introduction & Objectives:** Small ruminants play an important role in the transmission of verotoxin-producing *E. coli* (VTEC) to humans, and non-O157 VTEC serotypes isolated from ovine were associated with case of HUS. Sheep harbor different serotypes of VTEC in their gastrointestinal tracts, and previous studies suggest an animal-host serotypes specificity for some of those. The aim of this study was to evaluate VTEC prevalence in sheep, and to investigate VTEC serogroups by molecular methods.

**Material & Methods:** A total of 380 specimens (gut mucosa, skin, and carcass surfaces) from 50 sheep and 45 lambs, slaughtered in Sardinia, were examined by a direct preliminary PCR to detect vtx genes. Positive enrichments were submitted to the manual immunomagnetic separation (IMS) to detect the serogroups O157, O26, O103, O111 and O145. Then, each suspension was streaked onto the selective media, and typical colonies were identified by API system. The isolates were analyzed by multiplex PCR for stx1, stx2 and eae gene presence. A selection of 37 isolates from 34 specimens was submitted to: (i) a real time PCR, for the detection of O157, O26, O103, O111 and O145 (respectively: per, wzx, wzy, wzb1 genes); (ii) two PCRs, respectively for the O91 (wbsD gene) and O146 (wzx and wzy genes) serogroups.

**Results:** Basing on the preliminary screening, the overall prevalence of VTEC resulted 11.1% (14 sheep, 7.8 % lambs). In particular, 18.9% of the skin, 14.7% of the carcass surface and 10.5% of the mucosa samples were positive. On the basis of multiplex PCR results, the isolates belonged to three groups: VTEC: 43.4% (n.23), O103, O111 and O145: 26.4% (n.14), negative for all the genes (stx1-, stx2-, eae-). None of the 37 strains belonged to O157, O26, O103, O111, O145 and O146 serogroups, while n.21 (56.8%) to O91 serogroup.

**Conclusions:** Our results showed an occurrence of VTEC higher than that previously reported. The skin was confirmed as the major source of contamination, because of the faecal origin. The presence in the carcass surface was frequently correlated with visible faecal contamination, especially in lambs, which may have soft stool and sometimes diarrhea. Finally the results confirm the healthy sheep as reservoir of non-O157 VTEC serotypes, that showed virulence profiles potentially pathogenic for humans.
P-166
DNA Extraction and Molecular Detection Methods for Shiga Toxin-Producing Escherichia coli in Food
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Introduction & Objectives: The infections with non-O157 Shiga toxin (Stx)-producing Escherichia coli (STEC) are reported although serotype O157 is major. The transmitting vehicle, however, has not been identified in many outbreaks with non-O157 STEC. To detect Stx-producing E. coli in food, methods targeting Stx are necessary. In this study, we studied effective methods in consist from DNA extraction and DNA amplification methods to detect Stx-gene in food culture.

Material & Methods: Food samples (ground beef, ground beef containing 30% fat, cattle liver, cheese, egg, chicken with skin, pickles of cucumber, radish sprouts and alfalfa sprouts) were incubated in modified EC broth with novobiocin at 42°C for 20 h. STEC O26, O111 and O157 cultures in tryptic soy broth were 10-fold diluted to 10-2 in food culture. The food culture with STEC at various concentrations was used as samples for DNA extraction by boiling method, alkaline extraction method and three commercial kits. To detect stx gene, a loop-mediated isothermal amplification (LAMP) assay, three real-time PCR assays and conventional PCR assays were used.

Results: A boiling method was inferior to the other DNA extraction methods to detect stx in ground beef culture, and the limit of detection was more than 4.3 log cfu/mL of food culture. Most of the other DNA detection methods showed that the limit of detection was ranged from 2.1 to 3.7 log cfu/mL of food culture in most food samples. Stx detection in cheese by DNeasy followed by LAMP and conventional PCR assays was with the limit of detection of 4.4 and 4.1 log cfu/mL, respectively. LAMP and TaqMan PCR assays show similar sensitive results in various combinations with DNA extraction methods and food samples. In comparison with ground beef containing 30% fat, cattle liver and egg, a conventional PCR method with DNA extracted by alkaline extraction method was inferior to the other molecular detection methods, and the limit of detection was ranged from 4.1 to 4.6 log cfu/mL.

Conclusions: All of DNA extraction methods except for boiling method were successful to detect stx in most food samples by most of molecular detection methods with the limit of detection of <4.0 log cfu/mL of food culture. Molecular detection methods would be effective to detect STEC rapidly and sensitively.

P-167
The Incidence of Feedlot Cattle Shedding High Levels of Escherichia coli O157:H7
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Introduction & Objectives: The term ‘super shedder’ has been applied to cattle that are high shedders of Escherichia coli O157:H7. These animals are estimated to account for >80% of the E. coli O157:H7 shed in feces of cattle. We have hypothesized that the super shedding condition is a result of intestinal imbalance and that a healthy normal microbiota prevents E. coli O157:H7 colonization of the cattle intestinal tract. As a first step in testing this hypothesis, we examined the incidence of super shedder cattle in a commercial feedlot with the intention of profiling the fecal microbial communities of these individuals by pyrosequencing.

Material & Methods: Fecal samples were taken from 400 mixed breed feedlot cattle and enumerated for E. coli O157:H7 on Cefixime Tellurite Sorbitol MacConkey Agar. All samples were subjected to immunomagnetic bead separation and latex agglutination to confirm the presence or absence of E. coli O157:H7. Five non-sorbitol fermenting colonies from super shedder samples were collected and confirmed as E. coli O157:H7 by latex agglutination and multiplex PCR, and stored at -80°C. Pulse Field Gel Electrophoresis (PFGE) was used to genotype E. coli O157:H7 isolates.

Results: A total of 46/400 (11.5%) cattle were identified as shedding E. coli O157:H7; 11 (23.9%) of these animals were super shedders: animals shedding >1.0 x 104 E. coli O157:H7 CFU/g of feces. E. coli O157:H7 counts for the super shedder animals ranged from 1.5 x 1010 to 6.5 x 1010 CFU/g. PFGE analysis of the E. coli O157:H7 isolates from the super shedders showed a high degree of relatedness. Five super shedders and five negative control animals were slaughtered at 4 (one super shedder and one control), 8 and 10 (two super shedders and two control each day) days after the initial sampling. While all five super shedders remained positive for E. coli O157:H7, only the animal slaughtered on day 4 shed E. coli O157:H7 at >1.0 x 104 CFU/g.

Conclusions: In this study, nearly 25% of the animals shedding E. coli O157:H7 were super shedders and this state seemed to be produced by one to several closely related strains. The super shedding state appears to be short lived. Further studies are required to perform a more detailed characterization of the super shedding state and the contributing factors. Fecal samples collected from the slaughtered animals are currently being subjected to phylogenetic analysis to determine if the microbiota has a role in the super shedding phenomenon.

P-168
Changes in United States Regulatory Policy on Non-O157 STEC in Beef Products
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Non-O157 STEC are considered to be emerging pathogens worldwide. Incidence of non-O157 STEC related illness has increased in the U.S., partially through enhanced surveillance, diagnostic testing capacity, and awareness. Although illnesses have rarely been associated with beef in the U.S., outbreaks in Europe and Australia have been linked to beef or other meat products. USDA-FSIS is the public health regulatory agency responsible for ensuring that meat, poultry and egg products are safe to consume and properly labeled. FSIS inspectors verify the proper application of HACCP systems in regulated establishments which necessitates the identification of food safety hazards reasonably likely to occur in a product, as well as the measures taken to control the identified hazards. In 1994 after a large multistate outbreak, FSIS declared its intent to consider E. coli O157:H7 as an adulterant in ground beef, and initiated a testing program designed to verify that ground beef producers were properly controlling this hazard in their finished product. In 1999, the FSIS focus was expanded to include beef trimmings and other components used to produce ground beef and in 2002, the FSIS focus was expanded to include other non-intact beef products (needle or blade tenderized beef products). The actions taken by FSIS had a profound effect on the industry, resulting in reduced levels of E. coli O157:H7 contaminated ground beef and illness incidence. In September 2011, FSIS similarly declared its intent to consider non-O157 STEC as an adulterant in non-intact beef and its components,
and will initiate a testing program in June 2012. FSIS developed a non-O157 STEC policy after considering available scientific data, including questions used by FSIS officials in 1994 to justify the Agency’s position that *E. coli* O157:H7 was an “unusual and urgent food safety problem:

1. What are pathogenic non-O157 STEC, and how can they be distinguished from other STEC?
2. Are pathogenic non-O157 STEC present in cattle and beef, including ground beef?
3. Would traditional and accepted cooking practices for raw ground beef kill pathogenic non-O157 STEC?
4. Can small numbers of pathogenic non-O157 STEC cause illness?
5. Can pathogenic non-O157 STEC cause severe illness including permanent life-threatening damage to major organ systems?
6. Can pathogenic non-O157 STEC spread from person to person causing illness in settings such as day care facilities?

FSIS published a ‘risk profile’ describing its assessment of scientific data related to these questions. We will present FSIS’ assessment and thought process, as well as the new FSIS policy and testing program.

**P-172**  
**Prevalence and Molecular Epidemiology of *Escherichia coli* O26 in New Zealand Slaughter Cattle**  
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**Introduction & Objectives:** *Escherichia coli* O26 are a diverse serogroup including both Shiga toxin-producing *E. coli* (STEC) and Enteropathogenic *E. coli* (EPEC). In contrast to the mild diarrhoea associated with O26 EPEC strains, human STEC O26 infections can result in severe disease such as haemorrhagic colitis and haemolytic uraemic syndrome. Recent studies have identified both stx-positive and stx-negative O26 in New Zealand ruminants, therefore this larger and co-ordinated national study was undertaken to establish the prevalence of O26 from cattle and to undertake molecular typing to determine zoonotic potential.

**Material & Methods:** Faecal samples were taken from very young (4–7 days old) calves and adult cattle at 2 North Island (NI) and 2 South Island (SI) slaughter plants. RT-PCR (wzx O26) was used to identify O26-positive enrichments. Isolates were recovered using immunomagnetic separation and cultured on rhamnose MacConkey agar. Each O26 isolate was screened using multiplex PCR (stx1, stx2, eae, ehxA), and genotyped by pulsed-field gel electrophoresis (XbaI-PFGE). Stx-encoding bacteriophage insertion (SBI) typing was also used to genotype a subset of O26 isolates.

**Results:** Using RT-PCR from enrichment cultures, O26-positive enrichments were more prevalent from calves (33.2%) compared to adult cattle. Similarly, O26 strains were more commonly isolated from calves (8.6%) compared to adult cattle (3.2%). Most STEC O26 were only rarely isolated from adult cattle (0.5%) but were more frequently associated with calves (4.0%). Most STEC O26 (28/29) were stx1-, eae- and ehxA-positive; one isolate was stx1-, eaeA-positive and another was eae- and ehxA-positive. None of the 26 isolates possessed the vtx2 gene. Among them, 19 vtx2-positive isolates were identified as O91 serogroup. Furthermore, all isolates were O26 serogroup and were known as an important cause of severe illness in humans. Cattle are known to be one of the main reservoirs of VTEC. The microorganism is carried as a part of the native microbiota in the intestine and can contaminate carcasses when animals are slaughtered. Several serotypes of VTEC have been isolated from cattle; however, serogroup O157 is one of the most common groups of these pathogenic bacteria that originate from animals. The aim of the present study was to identify and characterize the VTEC isolates from the hides and the corresponding carcasses of cattle slaughtered in Poland, and assess their potential risk to human health.

**Material & Methods:** A total of 406 cattle slaughtered in three different abattoirs in eastern Poland during the period of 2008–2010 were examined for this study. Samples were collected from the hides before they were removed after exsanguination, using sterile swabs wiped across an area of 400 cm² of the brisket. The same sampling site and method were used for the carcasses of cattle after exsanguination. The detection and identification of VTEC was performed using the ISO 16654 standard and the PCR method as described by Wieczorek et al. (2008). The vtx1, vtx2, eaeA, and ehxA virulence genes were identified by PCR (Tatarczak et al. 2005).

**Results:** Thirty-two of the 406 bovine hides tested (7.9%) were contaminated with VTEC, and 24 of the corresponding 406 carcasses (5.9%) were positive for VTEC. The isolates were serotyped using the PCR method and only one of hide origin was identified as O91 serogroup. Furthermore, four VTEC identified on bovine carcasses were determined as O91 (three strains) or O145 (one isolate). None of the 56 VTEC identified during the study was of O157 serogroup. All VTEC were further characterized using the PCR method for the presence of the main virulence marker genes. Most of the isolates (51 out of 56; 91.1%) possessed the vtx2 gene. Among them, 29 vtx2-positive strains were isolated from hides (90.6%) and 22 strains from carcasses (91.7%). It was found that 24 VTEC possessed the vtx2c variant, four strains were vtx2d-positive and nine strains had the vtx2e gene marker. Many VTEC possessed also two additional virulence marker genes, encoding intimin (eaeA gene: 32.1% of the strains) and enterohemolysin (ehxA gene: 47.2% of the isolates).

**Conclusions:** The results of the present study indicate that beef meat may be a source of VTEC potentially pathogenic for humans. The
identified verotoxin-producing *E. coli* possessed several virulence marker genes that may cause human diseases. Furthermore, serotypes different that O157:H7 may be isolated from beef chain, therefore efforts should be made to routinely identify these kind of VTEC in food of animal origin.

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**P-175**

**Analysis of Adherence and Biofilm Formation by O157 and Non-O157 Shiga Toxin-Producing *Escherichia coli* Strains Isolated from Cattle Hides and Carcasses**

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Introduction & Objectives: Shiga toxin-producing *Escherichia coli* (STEC) is an important food-borne pathogen, and ruminant animals are considered to be the major reservoir. The ability to form biofilms may facilitate the maintenance of STEC on the animal reservoir and the bacterial spread in the environment. The aim of this study was to evaluate the ability of O157 (n = 14) and non-O157 (n = 8) STEC strains isolated from cattle hides and carcasses to adhere and form biofilm on biotic and abiotic surfaces.

Material & Methods: Adherence assays were performed in 96-well polystyrene microtiter plates, glass tubes and HeLa cells. Occurrence of fimH, flu, sab, cah, ehaA-alfa and ehaA-beta genes encoding some adhesins and autotransporter proteins was investigated by PCR. Detection of type 1 fimbriae (F1), curli, and cellulose was carried out through mannose-sensitive hemagglutination, CFA-Congo Red Agar, and Calcofluor Agar assays, respectively.

Results: Biofilm formation (BF+) was identified in only one O157 STEC strain at 28 and 37°C in polystyrene microtiter plates. Fourteen strains (63.4%), including six O157 and all non-O157 isolates, formed visible clumps as a ring in the glass tube wall at the broth surface. All O157:H7 strains presented a localized-adherence-like (LAL) pattern to HeLa cells, and in addition two isolates also showed an aggregate adherence to cells. Among the non-O157 STEC isolates, diffuse (n = 3) and diffuse/aggregative (n = 3) adherence patterns were detected, and two strains were non-adherent. The O157 BF+ strain also produced clump in glass tube, but no expression of the adhesins investigated was observed, thus suggesting that other structures may be involved in its ability to adhere to abiotic surfaces. Expression of curli and F1 occurred in 62.5% and 87.5% of the non-O157 isolates respectively, and 36.4% of them also produced cellulose. All strains presented cah, fimH, ehaA-alfa and ehaA-beta genes. However, only non-O157 STEC strains carried flu (36.4%) and sab (4.5%).

Conclusions: These results suggest that F1, curli and cellulose may be involved in the ability of some non-O157 strains to adhere to abiotic surfaces like glass, but further studies are necessary to confirm this hypothesis. The non-correlation observed between the ability of O157 STEC strains to adhere to the abiotic and biotic surfaces studied indicates that distinct mechanisms and structures should be participating in these processes.

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**P-176**

**Differences in Virulence Genes Frequency among VTEC Isolates from Cattle, Foods and Environment**

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Introduction & Objectives: The aims of this study were to assess the prevalent virulence genes and their combinations among a collection of VTEC isolates obtained from bovine cattle, foods and the environment, and to compare the results among isolates from different origins: calves, grown calves, adult cattle, foods and environment.

Material & Methods: The isolates had been obtained from bovine cattle (451 isolates comprising: 95 from calves, 75 from grown calves, and 281 from adult cattle which include dairy cows, feedlot cattle and cattle at slaughter), meat (74 isolates) and the environment of dairy farms (20 isolates). They were characterized by a multiplex PCR that detects vt1, vt2, eae, ehxA and saa.

Results: The vt2 gene was present in average in 85.1% of the isolates, followed by ehxA and saa genes (63.7% and 35.6%, respectively). When the isolates were grouped according to the origin, some marked differences in gene frequency were detected. The eae gene was detected in a high frequency in isolates from calves and the environment (74.7% and 65%, respectively), whereas the frequency was <15% in isolates from the other origins. The saa gene frequency showed an inverse trend, but never reached levels higher than 50%. The percentage of vt1-positive isolates was 24% among those from calves and the environment, and lower in the isolates from the other origins. The predominant virulence profiles, which comprised 78% of the isolates, were vt2, vt1, ehxA/saa, vt1/vt2/ehxA/saa, and vt2/eae/ehxA, arranged in decreasing order. Among calves, the profiles vt1/eae/ehxA and vt1/eae/ehxA and vt1/eae/ehxA were the most frequent, followed by vt2, and these three profiles also predominated among environmental VTEC isolates. The profile vt2 was the most frequent among grown calves, adult cattle and food isolates, followed by vt2/ ehxA/saa and vt2/vt1/ehxA/saa.

Conclusions: The frequencies of the genes detected in this study kept close similarity among isolates from meat, adult cattle and grown calves, differing from those from calves and the environment, which showed a higher frequency of eae and vt1 than the others. The relationship between VTEC isolates from meat and adult cattle is not unexpected as meat can become contaminated with VTEC during slaughter and the samples from adult cattle included cattle at slaughter and feedlot cattle that were near finishing. Besides it, on the other hand it can be speculated that young animals (calves) make an important contribution to the contamination of the environment in dairy farms.

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**P-177**

**Occurrence of Shiga Toxin-Producing *Escherichia coli* (STEC) in a Bavarian roe deer Population Over Different Periods of Time**

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Introduction & Objectives: Especially ruminants pose a risk for STEC infections in humans since they are regarded as the main reservoir. Game meat and therefore deer is described as important source, as well. Since there is no information about the duration of STEC excretion in game, this study deals with the occurrence of STEC in roe deer (*Capreolus capreolus*) at different periods of time. Additionally, obtained isolates will be characterised for serotypes, different virulence genes, PFGE-Types, and antimicrobial susceptibilities.

Material & Methods: Within the frame-work of a telemetric marking of roe deer, in February and March 2010, faeces samples from 20 roe deer were collected in the Bavarian Forest National Park. From one animal samples were obtained on two different days. From November 2010 until March 2011 rectal swabs from 35 roe deer were taken from the same population. From four and three animals swabs were collected on three and two different days, respectively. From four animals one sample was collected in each period. The next sampling period will be winter 2011/2012. Until now, 67 samples of 55 roe deer were taken. Due to organisational reasons the faeces samples from the first period were frozen at -20°C, while the swabs were placed in 10 mL buffered peptone water and refrigerated until analysis. The samples were analysed for the occurrence of the stx 1 and 2 using real-time PCR.

Results: In the first period 80% (16/20) animals had stx2-positive faeces samples. In winter 2010/2011 80% (28/35) animals had stx-positive rectal swabs, also. One of these samples was positive for stx1. From 12 animals, 2–3 samples each were obtained in different time intervals (from 4 up to 347 days). Eight of these animals were stx-positive in every sample (from 4 up to 347 days), two animals remained stx-negative about 17 and 69 days, respectively. In two cases the first sample was stx-negative and the second sample was stx-positive after periods of 50 and 314 days, respectively. The characterization of the isolates has not been finished yet, but preliminary results show a predominating stx-subtype (stx2b).

Conclusions: This study demonstrates the frequent occurrence of stx in roe deer faeces and rectal swabs. Due to the low number of animals sampled repeatedly so far, evidence for a long-term excretion of STEC in roe deer is still preliminary. Hence, the repeated sampling of further animals will be continued, and the characteristics of the isolated strains will be presented.

P-179
Development of an All-Ireland Food Microbial Database – A Risk Assessment Tool for VTEC Prevelance
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Introduction & Objectives: Verocytotoxigenic *Escherichia coli* (VTEC) are highly pathogenic zoonotic agents, characterised by the presence of verotoxins (vt1 or vt2 and their variants). They have the ability to cause illness in and severe cases cause death. Ireland has the highest per capita incidence (5.7 VTEC cases per 100 000) in Europe. The EU Zoonoses Directive (2003/99/EEC) mandates that all EU member states must provide data on zoonoses and zoonotic agents. In Ireland, all zoonotic data regarding VTEC is generated from institutional veterinary, food, public health and research laboratories, but at present there are no established links between these laboratories. One of the aims of this project is to link these laboratories by collating all VTEC data generated from Irish laboratories into one VTEC database. This project brings together established VTEC experts in research and regulation, to develop and implement a comprehensive programme designed to capture and analyse VTEC data. The main objectives are: (i) to develop and maintain a state-of-the-art all-Ireland food microbial database and to populate it with validated VTEC data from Irish data providers to provide relevant, reliable and up-to-date data in a format that allows timely decision-making, risk assessment and management; (ii) to develop an inaugural standardised molecular sub-typing database to include the four main clinically significant food-related microbial pathogens (*VTEC, Salmonella, Campylobacter and Listeria*); and (iii) to investigate the feasibility of linking Northern Ireland VTEC data, methods and reporting systems to the database, so as to provide an accurate all-island overview underpinning effective and efficient responses to challenges posed by VTEC.

Results: UCD is currently working with the other project partners to develop a web-based database (http://microbialdatabase.ie/). Database development is in the final phase and is on target to be fully operational by the end of 2011. The database will provide valuable information on VTEC recovered from clinical, food and environmental sources in Ireland covering the period 2009–2012 and beyond. Project partners and other key stakeholders will have full access to the database. It is envisaged that the database will set a benchmark standard at international level for microbial database systems, strengthen links between existing all-island institutional surveillance systems, improve data quality, identify data gaps and foster international links.

Conclusions: For the first time, an all-Ireland food microbial database will allow researchers and regulators access to and risk assessment of validated VTEC data from Irish data suppliers spanning a wide spectrum of diverse sample matrices from farm to fork.
and StcE<sub>O103</sub>, and Group B (espK positive), associated with stx1a. Although the stx genes were more frequently present in isolates from patients (52%) than from sheep flocks (5%), more than half of the ovine isolates in the EHEC/EHEC-like group had MLVA profiles identical to those seen in stx positive human O26:H11 isolates. It is likely that EHEC-like ovine isolates may be able to acquire stx-encoding bacteriophages and thereby cause serious illness in humans. The remaining one-third of the sheep flocks and two of the patients had eae<sup>+</sup> E. coli O26:H11 isolates fulfilling the criteria for aEPEC: stx negative, arcA allele type 1 and espK negative (Group C). Furthermore, these isolates lacked ehxA and they fermented rhamnose and dulcitol. The majority of the ovine isolates in this group showed MLVA profile 5-3-0-8-3-x-x, not previously seen in E. coli O26:H11 isolates from humans. However, according to their virulence gene profile (eae, nleB, efa1, lpfA<sub>1414</sub>, and lpfA<sub>O113</sub>), the ovine aEPEC O26:H11 isolates should be considered as potentially pathogenic for humans.

Conclusions: In conclusion, sheep is an important reservoir of potentially human pathogenic E. coli O26:H11 isolates in Norway.

P-180
Shigatoxigenic Escherichia coli in Synanthropic Rodents of the Metropolitan Area of Buenos Aires City, Argentina

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Introduction & Objectives: Shigatoxigenic Escherichia coli (STEC) is identified as one of the main pathogens that cause hemolytic uremic syndrome (HUS). This syndrome is an emerging endemic disease in Argentina which affects mainly children under the age of 5 and have a mortality rate of 5%.The aim of this study was to determine the role that synanthropic rodents from Buenos Aires Autonomous City (CABA) have on the epidemiology of HUS in the metropolis.

Material & Methods: One hundred forty-six wild and commensals rodents from CABA were captured using live capture traps. Two rectal swabs were taken from each animal for microbiological diagnosis. The samples were inoculated in 5 mL of TS (tryptone soya broth) with and without cefxime and tellurite (CT) for enrichment and incubated overnight at 37°C. After incubation, the cultures were streaked onto MacConkey agar and MacConkey Sorbitol Agar-CT and incubated overnight. Screening for stx1/stx2 and rfbO157 was done by Multiplex PCR from the confluence zone. STEC isolates were further characterized by biochemical tests by standard methods. Additional virulence factors (saa, eae and ehxA) were also determined by PCR. Forty-one of the rodents were necropsied and sampled from kidney and small and large intestine for histopathological diagnosis. The samples were fixed (10% buffered formalin), then they were cut (5 μm thick), and the sections were stained with hematoxylin-eosin, and observed by light microscopy.

Results: Twenty-seven from 146 animals were suspects (18%), obtained in isolation in seven cases: Rattus rattus (4/28), Rattus norvegicus (2/32) and Mus musculus (1/66). The following stx genotypes were found in the STEC strains: stx2 (4), stx1 stx2 ehxA (1), stx2 ehxA (1), stx2 ehxA eae (1). In the sample of 41 rodents necropsied five animals harboured STEC strains which were isolated, four animals were positive to the screening for STEC and 32 animals did not carry STEC strains) no macroscopic and microscopic lesions compatible with those produced by Shiga toxin were observed in the studied organs.

Conclusions: It has been observed that several species of urban rodents are carriers of STEC and it has been detected in the carrier state species with a shared habitat. There is no evidence of injury in these animals by those means of infection that are naturally exposed to. The absence of lesions in the kidney and intestine could be due to individual factors. These synanthropic species may play role in the transmissibility of this agent thus being a risk to the susceptible population, contaminating food and keeping the agent in the metropolis so should therefore be taken into account in health interventions before sources of infection and also in control programs of HUS.

P-181
Shiga Toxin-Producing Escherichia coli Isolated from Different Reservoirs Between 1998 and 2010 in Argentina

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Introduction & Objectives: Shiga toxin-producing Escherichia coli (STEC) is the most important emerging food-borne pathogen, and a major cause of gastroenteritis that may be complicated by hemorrhagic colitis, or hemolytic uremic syndrome. As it has subsequently been found that healthy cattle can harbor the bacterium, ruminants are now regarded as its main reservoir, though STEC has been isolated from other animal species such as sheep, pigs, geese, gulls and pets. Transmission occurs through the consumption of undercooked meat, unpasteurized dairy products, and vegetables or water contaminated by animal feces. In Argentina, where HUS is endemic, approximately 400 new cases are reported annually. STEC O157:H7 is the predominant serotype isolated from clinical cases, but little is known about the dominant subtypes in the reservoir. The aim of this study was to characterize STEC strains from different animal reservoirs from 1998 to 2010.

Material & Methods: A collection of 698 STEC and non-toxigenic O157 strains isolated from cattle (n = 625), dogs (n = 22), wild ruminants (n = 21), sheeps (n = 11), goats (n = 4), rabbits (n = 8), cats (n = 4), chinchillas (n = 2) and seagull (n = 1) during surveys and research programs, were included. The strains were characterized by biochemical tests, serotyping, stx-genotyping, and pulsed-field gel electrophoresis (PFGE) using the 24-h PulseNet standardized protocol.

Results: The strains were grouped into 45 serogroups and 82 serotypes. The prevalent serotype was O157:H7 (18.1%) followed by O178:H19 (8%), ONT:H2 (6.6%), O8:H19 (6.2%), O130:H11 (4.7%), O103:NM (4%) and O113:H21 (3.6%). Other serotypes involved in human disease, such as O111:NM, O121:NM, O145:NM, O174:H21, O26:NM and O91:H21, were also found. STEC O157:H7 strains were isolated from cattle, rabbits, dogs and sheeps. The stx-genotyping showed that stx2 (64.3%) was prevalent, followed by stx1 and stx2 (22.3%), and stx1 (10.7%). Eighteen (2.6%) strains were non-toxigenic and 11 of them isolated from dogs belonged to O157:H16 serotype. Among the 126 STEC O157 strains, 54 XbaI-PFGE patterns were identified with at least 61.2% similarity. Ninety-four isolates were non-toxigenic and 11 of them isolated from dogs belonged to O157:H16 serotype.

Conclusions: It is important to recognize that, though cattle are the main reservoir of STEC, other animals can harbor STEC. This
should be considered in order to take different prevention measures when handling these animals.

**P-182**

**Isolation and Characterization of Shiga Toxin-Producing *Escherichia coli* and *Citrobacter freundii* Strains from Ground Beef in Different Vulnerability Areas of Buenos Aires, Argentina**

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Introduction & Objectives: Consumption of raw/undercooked ground beef is the most common means of transmission of Shiga toxin-producing *E. coli* (STEC), especially O157:H7. The microbiological quality of ground beef could vary due to the socio-cultural characteristics of the population. The aim of this study was to evaluate the rate of STEC contamination in ground beef collected from different areas of Buenos Aires City, Argentina, according to families percent of unsatisfied basic needs, and to determine the virulence profiles of the strains.

Material & Methods: A total of 264 ground beef samples randomly purchased from small family butchers in three vulnerability areas with low, medium and high income were examined. The samples were cultured in Tryptic Soy Broth (TSB) followed by streaking onto MacConkey agar (MAC) and TSBm + Casaminoacids followed by IMS and plating onto Sorbitol MAC - CT(SMAC) agar plates. Confluent zones from MAC and SMAC were screened for STEC by PCR IMS and plating onto Sorbitol MAC - CT(SMAC) agar plates. Up to 50 individual colonies were further re-tested by PCR from positive plates. All stx+ strains were subjected to biochemical tests, serotyping, cytotoxic assay, production of enterohemolysin, and diffusion susceptibility test. Additional virulence genes, eae, eahX, and saa, were searched for all STEC strains. Statistical analysis was carried out using the difference in proportions test.

Results: Strains harboring the stx gene were isolated from 20.8% (55/264) of the samples. Eleven out of 55 samples (20%) contained O157strains. Among the 59 stx+ strains, 58 (98.3%) were *E. coli* (STEC) and one (1.7%) was *Citrobacter freundii*. Among the 22 different STEC serotypes, O178:H19 was the prevalent one. A high rate of contamination by O157:H7 was seen in ground beef purchased from the area with medium and high income (P < 0.05). The STEC strain frequencies of virulence markers corresponded to: stx1 (3.57%); stx2 (89.29%); stx1 plus stx2 (7.14%); eahX (56.9%); eae (27.6%); and saa (25.9%). Furthermore, 94.6% of the strains were cytotoxic, and 42.9% were EHEC-Hly+. The *Citrobacter freundii* virulence profile corresponded to stx2 eae. The STEC strains showed resistance to ampicillin (AMP) (14%), streptomycin (S) (14%), amikacin (AK) (1.8%) and tetracycline (TE) (1.8%). Additionally, STEC strains showed reduced sensitivity to S (40%), TE (9%), AMP (5.2%) and nalidixic acid (3.6%). β-lactamases and carbapenemases were not detected.

Conclusions: Contamination of meat with STEC was here related to vulnerable areas. To improve the quality of meat, the cultural patterns and the perception of risk by the food handler and the community should be analyzed. Since 78.9% of the strains studied in this work were non-O157 STEC, it is suggested that criteria should be developed and methods should be validated to facilitate their identification and control.

**P-183**

**Verocytotoxigenic (VTEC) and Enteropathogenic (EPEC) *Escherichia coli* Presence in Chicken and Retail Products from the Farm to the Market**

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Introduction & Objectives: Verocytotoxigenic (VTEC) and enteropathogenic (EPEC) *Escherichia coli* are important pathogens related to Public Health. VTEC cause hemorrhagic colitis and hemolytic uremic syndrome in humans. EPEC produce potentially fatal infant diarrhea noticeably in developing countries. Most of the studies in chicken and retail products were focused on O157:H7 VTEC, but there is scarce information about non-O157 VTEC and EPEC contamination. The aim of this study was to evaluate the proportion of chicken samples contaminated with VTEC or EPEC in live animals and their products (carcasses, giblets and hamburgers).

Material & Methods: Cloacal swabs from live animals (859) were taken from three farms and a chicken processing plant. Carcasses swabs (457) were obtained from different shops and from washed carcasses after the chilling step in the processing plant. In addition 300 giblets and 300 hamburgers were sampled from different shops. All the samples, after an enrichment step, were analysed by a multiplex PCR to detect vt1, vt2 and eae genes.

Results: The carriage of VTEC was very low in live animals but notably increased during processing and handling especially in chicken hamburgers (Fig. 1). Regarding EPEC, proportion of positive samples was higher than VTEC and showed a more gradual increase.

Conclusions: These results show that EPEC and specially VTEC contamination increase after processing and handling of chicken products, and reinforce the need to improve control measures along the production and marketing.
Prevalence and Virulence Profiles of Shiga Toxin–Producing Escherichia coli Isolated from Beef Cattle in a Brazilian Slaughterhouse

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Introduction & Objectives: Shiga toxin-producing Escherichia coli (STEC) are responsible for severe human diseases. Cattle are the main STEC reservoir, and meat and meat products have been implicated in disease outbreaks worldwide. Brazil represents one of the largest exporters of beef, and few researches have been done searching for STEC in beef carcasses. The aims of this study were to evaluate the occurrence of STEC in a processing plant that produces bovine meat for export, determine the virulence characteristics and the genetic diversity of the isolated strains.

Material & Methods: Surface samples were collected from 400 animals at three stages of the slaughtering process: hide right after bleeding (H), carcass after hide removal (C1), and carcass after cleaning but before chilling (C2). Samples were enriched and submitted to immunomagnetic separation (IMS) for O157. A multiplex PCR to detect stx1, stx2 and eae genes was also carried out, and stx positive samples were further submitted to IMS for O26, O103, O111 and O145 serogroups. Isolates were confirmed as E. coli, serotyped, and tested for several accessory virulence markers. Antibiotic resistance profiling and PFGE analysis were also performed.

Results: The multiplex PCR screening showed that 187 of the 400 (46.7%) animals tested positive for stx, but STEC strains were only recovered from 94 (50%) of them. Isolation of O157 and non-O157 STEC strains occurred in 75 (18.7%) and 22 (5.5%) of the animals, respectively. In general, more than 90% of the animals tested positive for STEC only in hide, and just a few animals presented STEC both in the hide and C1 point (n = 7) or only at the C1 point (n = 2). Eighty-four STEC strains belonged to serotype O157:H7; whereas a diversity of serotypes occurred among the 57 non-O157 STEC strains, but none of them belonged to serogroups O26, O103, O111 and O145. More than 55% of the STEC isolates harbored only stx2, and stx1/stx2 was more frequently observed among non-O157 O111 and O145. More than 55% of the STEC isolates harbored only stx2, and stx1/stx2 was more frequently observed among non-O157 O111 and O145. More than 55% of the STEC isolates harbored only stx2, and stx1/stx2 was more frequently observed among non-O157 O111 and O145. More than 55% of the STEC isolates harbored only stx2, and stx1/stx2 was more frequently observed among non-O157 O111 and O145.

Conclusions: This study highlights that, although present in animal hides, the O157 STEC isolation at beef carcasses was rare, certainly due to effective hygiene practices during processing. Nevertheless, the prevalence of STEC O157:H7 identified in cattle in this study was higher than previously reported in Brazil.
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Characteristics of Shiga Toxin Producing E. coli (STEC) Non-O157 Strains Isolated from Ruminants Between 2003 and 2011 in Uruguay
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Introduction & Objectives: STEC is an emerging zoonotic pathogen that causes outbreaks and sporadic cases of foodborne diseases like hemolytic uremic syndrome and bloody diarrhea (BD), worldwide. In our country, some sporadic cases of BD are associated with non-O157 STEC strains in children. Ruminants are the most important reservoir of human STEC strains. The aim of this study was to characterize the STEC strains isolated from ruminants in Uruguay to establish their potential pathogenic role in human disease.

Material & Methods: A total of 66 STEC strains were included in this study. Sixty-four were obtained from rectal swabs of 58 bovines and six sheeps during the period 2003–2005; the other two were isolated from beef products in 2011. The serotype was determined with the methodology previously described by Orskov and Orskov (1984). The stx1/2 subtyping was performed by PCR-RFLP, according to the methodology previously described by Pradet et al. (2008). PFGE was done among strains of the same serotype, to see their clonal relationships.

Results: STEC isolates belonging to 18 O serogroups and 20 O:H serotypes were identified in 58 of the 66 strains. The remaining eight strains were non-typeable. The most prevalent serogroup was O26 (16%), followed by O8 (14%), O22 (10%), O178 and O163 (9% each). Other serogroups found were O103, O111 and O113. The 20 serotypes included O26:H11 (16%) as the most prevalent one, followed by O8:H19 (12%), O22:H8 (10%), O163:H19 (9%) and O178:H19 (9%). A total of 14 strains (24%) belonged to serotypes previously associated with severe human disease: O103:H2, O111:NM and O113:H21. No strain belonged to O157:H7 serotype. Forty-four percent of the 66 strains belonged to stx2 genotype, followed by stx1/stx2 (39%) and stx1 (17%). The stx1 variants were mainly stx1a-EDL933 (71%), while the main stx2 subtype was stx2c-EDL933 (89%). The most frequent stx1/stx2 combination was stx1c-933/stx2-EDL933 in 21 of the 24 stx1/stx2 STEC strains (88%). Some strains belonged to highly virulent seropathotypes, like O26:H11 stx1c-EDL933 or stx2-EDL933, O103:H2 stx1c-933/stx2-EDL933 or stx2-EDL933, O111:NM stx1a-933/stx2-EDL933 (seropathotype B) and O113:H21 stx1a or stx2-EDL933 (seropathotype C), according to the classification of Karmali et al.

Conclusions: This is the first report that analyses the STEC strains serotypes isolated from bovine and sheep samples in Uruguay, showing that most of them were serologically and genetically diverse. Although most of these isolates did not correspond to serotypes associated to severe human disease, O26:H11, O103:H2, O111:NM and O113:H21 STEC strains were detected. Since O26 and O111 serogroups have been associated with severe disease in children from Uruguay, cattle appears to be an important reservoir of these highly virulent seropathotypes in this region.

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Characteristics of Shiga Toxin Producing Escherichia coli (STEC) O157:H7 Strains Isolated from Food and Animals During 2002–2011 in Uruguay
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Introduction & Objectives: STEC, an emerging zoonotic pathogen producing food-borne illness worldwide, may cause severe complications as the hemolytic uremic syndrome (HUS) in humans. Its main reservoir is the gut of cattle from which it contaminates water and food, being O157:H7 the most pathogenic and prevalent serotype described. The aim of this study was to compare the phenotypic and genotypic characteristics of STEC O157:H7 strains isolated from food and animals in Uruguay, during 2002–2011, establishing their genetic diversity and clonal relatedness.

Material & Methods: Samples of ground meat ready for sale and presumptive strains isolated from food and ruminants were studied. The meat was processed by immunomagnetic separation for O157 and cultivated in enrichment medium. Presumptive colonies were studied by rapid agglutination test with anti-O157 serum and confirmed by PCR with specific primers for stx1/2. All isolates underwent phenotypic identification by conventional biochemical tests, serotyping (H7) and antibiotic susceptibility assays. Different virulence markers were featured by PCR, subtyping the stx1/2 genes by PCR-RFLP. The clonal relationship was studied by PFGE and phage typing.

Results: A total of 42 STEC strains belonging to serotype O157:H7 were detected from both origins. Most of them were obtained from food 35/42 (83%): beef (15), ground meat (6), hamburger (3), ovine trimming (2) and non-defined foods (9); two non-toxigenic E. coli O157 strains were also detected from food. The other seven strains (17%) were isolated from bovine carcasses (6) and from a sheep leather sample (1). All the strains showed typical phenotypic features of O157 strains: sorbitol and β-glucuronidase (-), and biotype C except one from food origin (biotype D). All but one strains were susceptible to all antibiotics, including both non-toxigenic cultures. The O157:H7 serotype was confirmed by PCR with specific primers in all of them. The most prevalent genotype was stx1/stx2 (53%), followed by stx2 (28%) and stx1 (17%) with stx1-933f and stx2c(vha) as the most prevalent stx1 and stx2 subtypes, respectively. All the strains were eae-1 and ehaA positive; 47%, 37%, 34% of them were positive for the genes espF, katP, and tcpP, respectively. All the strains showed great clonal diversity.

Conclusions: All the STEC O157:H7 strains isolated from food and animals in Uruguay are EHEC strains of A seropathotype, with great potential pathogenic features. Their presence should not be underestimated despite showing low clonal relationship. We should divulge these findings and further investigate this pathogen to contribute to control and prevent its spread, given our role in protecting human health and our condition of quality beef producing and exporting country.
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Non-O157 STEC is the Most Prevalent Pathotype Among Street-Vended Food and Beverages in Mexico City: Four studies in 10 years
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Introduction & Objectives: Street-vended food (SVF) provides, especially in large cities of developing countries, including Mexico, employment and cheap ready-to-eat meals to a large proportion of the population. Consequently, in the last 10 years we assessed the microbiological status of street-vended food and beverages (SVB), in Mexico City (>20 million inhabitants).

Material & Methods: Four studies were conducted, dates and number of samples collected were: (i) February–August 2000, 178 taco dressings (chile sauces, raw coriander, onion and lettuce); (ii) May 2005, 48 seafood samples; (iii) September–December 2010, 56 beverages; and (iv) November 2010–July 2011, 70 seafood samples. From all samples, pH was determined and human enteropathogens determined (enterobacteria, Vibrio spp., Aeromonas spp.) by traditional methods. E. coli, was used as indicator of faecal contamination, briefly samples were plated on Mac Conkey agar, number of E. coli -like colonies was recorded, five colonies were selected and tested for indole production. Positive isolates were analysed by 2 multiplex PCR for diarrheagenic E. coli pathotypes (DEP) loci: for enterotoxigenic (st-heat stable, lt-heat-labile enterotoxins), typical and atypical enteropathogenic (eaeA-intimin, bfp-bundle-forming pilus), enteroinvasive (ial-invasive-associated loci), STEC (stx-1,2-Shiga toxin 1,2, eaeA) and enteroaggregative (aggR-master regulon, aap-dispersin, aatA-autotransporter Tol C). STEC isolates were further characterized for the O157-LPS antigen and enterohemolysine gene (hlyA), by a latex agglutination kit and PCR, respectively.

Results: In Mexico City, most SVF and SVB items are faecally contaminated; non-0157 STEC is the most frequently DEP identified which it is enough quantities to cause disease (Table 1).

Conclusions: Over the last 10 years non-O157 STEC continues to be the most frequently DEP identified in SVF and SVB, all non-O157 STEC isolates from beverages also harboured the intimin loci. Although, in 2005 STEC was not identified in seafood samples, this year samples from the same stalls harboured non-O157 STEC. Therefore, consumption of SVF and SVB are a potential risk for non-0157 STEC and EHEC infections for both the local population and international travellers visiting Mexico City.

Table 1. Faecally contaminated samples and characteristics of STECa positive samples

| Study, no. | samples | E. coli positive samples (%) | DEP positive samples (%) | DEP STEC positive samples (%) | Type of food (no. samples) | pH range | Gene profile (No. samples) | STEC range 103, food CFU/g, beverage CFU/L |
|-----------|---------|----------------------------|--------------------------|--------------------------------|----------------------------|----------|---------------------------|-----------------------------------------------|
| 1b, 178   | 120 (67)| 8 (7)                      | 6 (75)                   | Red chile sauces (4) coriander/onion mix (2) | 4–6                       | stx1-stx2 (4) stx2 (2) | 1.3–100                                    |
| 2b, 48    | 12 (25)| 0                          |                          |                                  | 6–7                       | stx1-eaeA (2) stx2-eaeA (1) | 2.8–8.3                                    |
| 3b, 56    | 26 (11)| 3 (5)                      | 3 (100)                  | Horchataa Fresh strawberry drink | 5–8                       | stx2 (2) stx1-stx2 (1) | 5.3–150                                    |
| 4, 70     | 24 (34)| 3 (4)                      | 3 (100)                  | Shrimp oysters                   |                            |          |                           |                                               |

aNone expressed the O157 LPS antigen.

bStudies have been published.

Horchata = a rice-based traditional beverage.

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subpopulation has been identified as highly vulnerable to VTEC infections and has been subject to severe consequences in a major VTEC outbreak occurred in South Australia between late 1994 and early 1995.

Conclusions: The proposed correlation between the high level of VTEC infections occurred in Australia during the summer season for the period of 2001 and 2010 and the increased frequencies of exposure to outdoor environment and consumption of cold meat over the summer period, if verified, will assist future public health education campaigns targeting the minimisation of exposure to sources of VTEC infections.

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Real-Time PCR Detection and Isolation of Verotoxigenic Escherichia coli O26, O103, O111, O145 and O157 from Slaughter Cattle Hides
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Introduction & Objectives: Cattle hides are important route of contaminating carcasses in slaughterhouses. The objective of the study was to assess real-time PCR based screening method for verotoxigenic Escherichia coli (VTEC) O26, O103, O111, O145 and O157 in slaughter cattle hides. The study was conducted in 2008 with minor deviations from the technical specifications given in the EFSA Guidance on surveys on VTEC in animals and food, EFSA Journal 2009, 7(11):1366.

Material & Methods: A total of 155 swab samples (five samples per day) were taken from the brisket area of cattle hides during slaughtering in September–October. The animals were <30 months of age. The samples were incubated overnight at 37°C in buffered peptone water (BPW) or modified tryptone soy broth with 16 mg/L novobiocin (mTSB + n) and DNA was extracted from the broth. The samples were screened for stx1, stx2 and eae genes with two different real-time PCR methods, an in-house PCR and a commercial GeneDisk Cycler assay. The O serogroup genes for O26, O103, O111, O145 and O157 were determined from the samples grown in BPW by conventional PCR and GeneDisk assay. VTEC was isolated with immunomagnetic separation (IMS) combined with colony hybridisation from the samples positive in stx, eae and at least one of the O serogroup PCR. The isolates were genotyped by PFGE.

Results: Out of the 155 samples, 147 (95%) were positive for stx (stx1 or stx2), eae and at least one of the O serogroup genes. There were 114 (74%) samples positive for stx1, 150 (97%) for stx2 and 152 (98%) for eae. The O serogroup genes for O26, O103, O111, O145 and O157 were determined from the samples grown in BPW by conventional PCR and GeneDisk assay. VTEC was isolated with immunomagnetic separation (IMS) combined with colony hybridisation from the samples positive in stx, eae and at least one of the O serogroup PCR. The isolates were genotyped by PFGE.

Conclusions: The prevalence of genes for verotoxigenic Escherichia coli O26, O103, O145 and O157 in cattle hides was high (95%) when studied from enrichment broths by real-time PCR. However, the presence of the VTEC strains could only be confirmed from few samples (7.7%) by isolating the strains using colony hybridisation. The high amount of initially PCR positive samples makes this method very laborious and not suitable for screening large sample amounts.

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Occurrence of Escherichia coli O145 in Norwegian Sheep Flocks
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Introduction & Objectives: This study is part of a large Norwegian survey of Escherichia coli in sheep investigating the occurrence of the five well known VTEC serogroups – O26, O103, O111, O145, and O157. VTEC are characterized with their ability to produce Shiga toxins (encoded by stx) and cause attaching and effacing lesions in the intestinal mucosa. The adherence of bacteria to the intestinal mucosa is mediated by intimin and is encoded by eae. Moreover, stx negative, but eae positive E. coli of the same serogroups as the well-known VTEC serogroups do exist in the animal reservoir. These may be precursors for STEC by bacteriophage infection or there could be that it exists two groups of these of which one group is similar and one more different to STEC. The aim of the present study was to explore the occurrence of E. coli O145 with different virulence characteristics in Norwegian sheep flocks.

Material & Methods: During autumn 2007, 50 faecal samples were collected from 499 randomly selected Norwegian sheep flocks. From these, a subsample of 149 flocks was randomly selected for the investigation of E. coli O145. The faecal samples were analysed as pools of 10 individual samples; i.e. five pooled samples per farm. A modification of NMKL method 164 where the IMS method was further modified to include an ELISA step was used for detection of E. coli O145. ELISA positive samples were plated onto selective agars for colony isolation. E. coli isolates were O-typed by conventional methods and with real-time PCR targeting wzy in the O-antigen cluster of O145. In addition, a real-time PCR targeting iph1 recommended by the Community Reference Laboratory (CRL) to recognize STEC O145 were included. Detection of the virulence genes stx1, stx2 and eae was performed by real-time PCR following the CRL recommendations, and genotyping by PFGE.

Results: Preliminary results show that no STEC O145 was detected. However, stx negative, eae positive E. coli O145 was detected from 29% of the flocks. A selection of these isolates was specifically checked for stx2 as this stx subtype is not covered by the ordinary stx primers. None of the isolates carried stx2f. All isolates were positive by the wzy PCR, but only some of the isolates originating from 16% of the flocks were positive by the human pathogenic STEC O145 specific iph1 primers. It could be questioned whether this may support the theory that it exists two groups of stx negative, but eae positive E. coli O145 in sheep, of which one group is similar and one more different to the human pathogenic STEC O145.

Conclusions: The study did not detect any reservoir of STEC O145 in sheep. However, there is a relatively large reservoir of stx negative, but eae positive E. coli O145 in sheep. More studies with in-depth characterization of animal isolates and comparison to human isolates are needed to evaluate the degree of association between this sheep reservoir and human disease.
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VTEC in Raw Cow’s Milk in Denmark

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Introduction & Objectives: The aims of this study were to evaluate real-time PCR assays for detection of VTEC and E. coli O157 in raw cow’s milk and use the assays to determine the prevalence of VTEC in Danish raw milk samples. An additional aim was to determine the quantitative levels of E. coli in milk samples.

Material & Methods: Milk samples of 25 mL were incubated in 225 mL of tryptic soy broth added 8 mg/L novobiocin for 16 h at 37°C. DNA was purified from 1 mL of enriched sample and analyzed for genes specific for vtx1, vtx2, eae, O157, O26 and O111 respectively, by real-time PCR assays using dual-labeled probes. Generic E. coli was enumerated using Petrifilm™ count plates from 3M™.

Results: The vtx and O157 PCR screening assays were validated by comparison with the standard spiked with 5–50 CFU of vtx1 and vtx2 positive Zoonoses and Public Health/C211/C211 2012 The Authors in Denmark.

Material & Methods: Milk samples of 25 mL were incubated in E. coli quantitative levels of in Danish raw milk samples. An additional aim was to determine the cow’s milk and use the assays to determine the prevalence of VTEC ent PFGE profiles. strains were further analyzed by PFGE and all strains exhibited different PFGE profiles. The results of the PCR assays were in concordance with the ISO standard; the 30 spiked samples were positive for O157 and the 30 non-spiked samples were negative. The 30 spiked samples were also positive for vtx1 and vtx2.

The PCR assays were used to investigate 312 milk samples from dairy farms. Sixty-one (19.6%) of the samples were positive for vtx (vtx1, 5.6%; vtx2 10.6% and vtx1 + vtx2, 3.5%). The eae gene was detected in 32.7% of the samples and 11.9% were positive for both vtx and eae. E. coli O157 specific genes were detected in 8.0% of the samples and 1.3% of the samples were both vtx and O157 positive. The vtx positive samples were analyzed for O26 and O111 specific genes; 3 samples were O26 positive whereas none of the samples were O111 positive.

In order to obtain VTEC isolates, each of the 61 vtx positive enrichment broths were seeded on TBX agar. Pools of colony material from 10 colonies were screened for vtx by the PCR. This approach yielded VTEC isolates from two samples; these were eae negative and of serotype O116:H- and O126:H20. E. coli O157 were isolated from 20 (6.4%) of the 25 O157 real time PCR positive enrichment broths. Strains were isolated by seeding the enrichment broths to blood agar followed by O157 agglutination tests. All 20 E. coli O157 strains were PCR negative for vtx. The strains were further analyzed by PFGE and all strains exhibited different PFGE profiles.

The quantitative levels (CFU/mL) of E. coli in the 312 samples of raw milk were <1: 22.0%, 1–10: 50.3%, >10–100: 21.3% and >100: 6.5%.

Conclusions: This study shows that real-time PCR assays are efficient for screening of raw cow’s milk for VTEC, but also highlights the difficulties in obtaining isolates from PCR screening positive samples. The results indicate that the prevalence of VTEC O157 is low in Danish milk, but genes encoding verocytotoxin and other VTEC associated genes are frequently found. The study also shows that non-pathogenic E. coli O157 prevails in raw milk in Denmark.

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First Isolation of Sorbitol-Fermenting Enterohemorrhagic Escherichia coli O157 in Minced Beef Responsible for Hemolytic and Uremic Syndrome in France in 2011

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Introduction & Objectives: In June 2011, at least 11 cases of Hemolytic and Uremic Syndrome (HUS) have been identified in the Nord-Pas-de-Calais and Picardie, north of France. Investigations have shown that this outbreak was due to the consumption of contaminated frozen minced beef. The objective of this study was to identify, characterize and compare strains isolated both from human cases and meat.

Material & Methods: Patient’s stool and blood samples were analyzed in order to confirm the EHEC infection either by isolating strains or by serology testing. Minced beef samples were analyzed to isolate both typical EHEC strains belonging to the five major EHEC serogroups (O26, O103, O111, O145 and O157) according to the ISO TS 13 136 for the detection of EHEC strains in foods, but also other EHEC which could be responsible for HUS cases. Strains isolated from humans and meat were serotyped and phenotypically characterized for sorbitol fermentation, β-D-glucuronidase activity and motility. Genetic characteristics were determined by PCR (eae and stx variants and spfA gene), PFGE, MLST and MLVA.

Results: More than 200 isolates from meat and 11 isolates from humans were identified and characterized. In meat samples, genetic characterization determined a total of five PFGE profiles of EHEC strains including two profiles of sorbitol-fermenting E. coli O157.Interestingly, strains isolated from humans and meat share the same phenotypic characteristics, virulence and genetic profiles, supporting the relatedness between meat and human isolates and the epidemiological evidence implicating meat as the source of outbreak.

Conclusions: Even if meat has already been implicated in human cases, it must be emphasized that in this case, samples were contaminated by at least five different EHEC strains. Strikingly, it is the first time that sorbitol fermenting EHEC O157[H7] strains, similar to the so-called ‘German clone’, are isolated in food. The reservoir of such strains that have already caused large outbreaks has never been identified so far. Yet, our results suggest that cattle may be one of the reservoirs of sorbitol fermenting EHEC O157.
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**Occurrence of Escherichia coli O103 in Norwegian Sheep Flocks**

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**Introduction & Objectives:** A national foodborne outbreak caused by *Escherichia coli* O103:H25 occurred in Norway in 2006. Fermented sausage containing sheep meat was identified as the source of the outbreak, although only six different *E. coli* O103:H25 was isolated from sausage and sheep meat. Subsequently, a nationwide Norwegian survey on *E. coli* in sheep was initiated during autumn of 2006.

One of the serogroups included in the survey was *E. coli* O103. The present study reports the identification, occurrence and genetic relatedness of STEC, enteropathogenic (EPEC) and non-pathogenic *E. coli* isolates of *E. coli* O103 belonging to different H-types.

**Material & Methods:** During autumn of 2006 and 2007, faecal samples were collected from a total of 585 randomly selected Norwegian sheep flocks. Fifty animals per flock were sampled and analysed as pools of ten individual samples; i.e. five samples per farm. The pooled samples were analysed by a specific AIMS-ELISA for *E. coli* O103. ELISA positive samples were plated onto selective agar for colony isolation. *E. coli* isolates were O- and H-typed by conventional serotyping, and identified as STEC, EPEC and non-pathogenic *E. coli* by virulence detection of stx, eae and bfpA by PCR. A selection of isolates was H-typed by PCR and sequencing of a part of the flc gene. Genotyping of EPEC, STEC and non-pathogenic *E. coli* O103:H2/H103:H25 was performed by PFGE, and the PFGE banding patterns were compared using a combination of visual inspection and BioNumerics version 6.1 software (Applied Maths NV, Ghent, Belgium).

**Results:** *Escherichia coli* O103 was detected from 184/585 flocks (31.5%). Most of these were identified as *E. coli* O103:H2 or O103:H25, but several other H-types were also detected. Atypical EPEC O103 was detected from 53 flocks (9.1%), and these were either of serotype O103:H2 (3.1%) or O103:H25 (6.0%). STEC were identified from only four flocks, and all of these were of serotype O103:H2. The *E. coli* O103 isolates were genetically diverse, though epidemiologically unrelated *E. coli* O103 isolates did have identical PFGE profiles.

**Conclusions:** To the author's knowledge, this is the first large study in sheep flocks focusing on *E. coli* O103 in general, and including results on STEC, EPEC and non-pathogenic isolates. The results show that although *E. coli* O103 isolates from sheep belong to different serotypes (H-types), only isolates belonging to *E. coli* serotypes O103:H2 and O103:H25 could be identified as STEC or atypical EPEC and thereby be considered as potentially pathogenic to humans.

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**Growth and Survey of the STEC in Raw Milk Cheeses**

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**Introduction & Objectives:** Shigatoxin producing *Escherichia coli* (STEC) are an important cause of several human illness outbreaks. They're responsible of gastrointestinal diseases such as diarrhea, hemorrhagic colitis (HC). In some dramatic cases, STEC can cause hemolytic-uremic syndrome (HUS) and death. STEC are part of the normal intestinal flora of lactating animals. Public health importance of STEC, presence in milk and dairy products remains unclear. In fact, the behaviour of STEC in raw milk cheeses is unknown: many parameters in cheese manufacturing and ripening processes could influence growth and survival of these bacteria. Consequently knowledge of the STEC behavior in cheeses would help to evaluate risk for human health. The aim of our study was to observe the growth and survival of experimentally inoculated STEC strains in raw milk cheeses manufactured and ripened according to different technological schemes.

**Material & Methods:** Five different types of cheese were prepared: two semi hard cheeses (SHC) and one hard cooked cheese (HCC) made with cow milk, one blue mould cheese (BMC) made with sheep milk, and one lactic soft cheese (LSC) made with goat milk. They were experimentally contaminated with between 2 and 4 different STEC serotypes (O157:H7, O26:H11, O103:H2 and O145:H28). These strains were chosen for their virulence profile (gene stx1 or stx2) and prevalence in milk products. They were inoculated artificially and individually in raw milk (around 10⁶ CFU/mL) before cheeses making. Their growth and survival were monitored on selective medium during the fabrication and ripening processes.

**Results:** Two STEC compartments were observed: In the two SHC and in the BMC, STEC growth was observed during the first steps of manufacturing. In SHC and BMC, ripened respectively 7 and 6 months, STEC concentration decreased during ripening. For this SHC, STEC O26:H11 was about 10⁶ UFC/g and STEC O157:H7 was <50 UFC/g. For BMC, only STEC O26:H11 was detected at the end of ripening. On the contrary, for the HCC ripened only 2 weeks, the concentration of all STEC serotypes tested remained constant around 10⁶ and 10⁵ UFC/g. For the HCC and the LSC, no STEC growth was observed during the first hours of cheeses manufacturing. During the ripening and the conservation steps, most of the STEC strains remained only detectable in the cheeses. We could hypothesized that for the HCC, and the LSC, the heating stage (35°C during 35 min) and the long acidic coagulation step (24 h at pH reached 4.5) respectively, could have an impact on STEC strains behavior. In addition, in each technology (excepted for HCC), the P-values observed showed a serotype effect whereas there was no significant effect of the 'strain' factor on the behavior of STEC O103:H2, O157:H7 and O26:H11 in cheese type SHC and LSC.

**Conclusions:** This project permits a better knowledge of the behavior of different serotypes of STEC in raw milk cheeses with various manufacturing and ripening processes.

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**Characterization of Escherichia coli O157 Isolated from Cattle and Meat Products in Tucumán, Argentina**

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**Introduction & Objectives:** *Escherichia coli* O157 is an important and emergent foodborne pathogen associated with diarrhea, hemor-
rhagic colitis, and hemolytic uremic syndrome (HUS). In Tucumán, the incidence rate of HUS is 4.1/100 000 children under 5 years old, and O157:H7 is the most prevalent serotype responsible for the majority of the cases. Several authors have described the association between human infection and consumption of contaminated beef products, and have demonstrated the role of cattle as the main reservoir of STEC O157:H7. The aims of this study were to characterize STEC O157 isolated from cattle and meat products collected at abattoirs and retail stores, and to establish the clonal relatedness among the isolates.

**Material & Methods:** Between 2009 and 2011, 32 carcasses, 60 ground beef and seven fresh sausages samples were analyzed. The swabs from four different carcasses areas of 100 cm², and 65 g of beef products were processed according to the methodology described by USDA-FSIS 2008. STEC O157 strains were phenotypically and genotypically characterized. The subtyping by pulsed-field gel electrophoresis (PFGE) was performed using the 24-h PulseNet E. coli O157 standardized protocol.

**Results:** STEC O157:H7 strains were isolated from 3/60 (5%) ground beef and 1/7 (14.3%) fresh sausage samples, and characterized as stx2(c,8a-)/eae/ehxA (three strains), and stx2/eae/ehxA (one strain). Non-toxigenic E. coli strains were also isolated from 5 ground beef samples and one carcass, serotyped as O157:NT (five strains) and O157:NM (one strain). The strains yielded 10 different XbaI-PFGE patterns. By comparison with the Virulence profile database of STEC and E. coli O157 strains, 2149 strains/897 XbaI-PFGE patterns, none of the patterns have been previously detected in strains of different origins.

**Conclusions:** STEC O157 strains were isolated from ground beef and fresh sausage samples at retail store level in Tucumán province, in the northwest region of Argentina. These findings could contribute to a better characterization of the STEC O157 contamination, providing data for risk assessment in meat products in this province.

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**P-199**

**Prevalent STEC Serotypes Isolated from Cattle, Foods and Environment in Argentina**

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**Introduction & Objectives:** VTEC is the main aetiologic agent of HUS and cattle are the main reservoir of VTEC that is transmitted to humans through contaminated foods, water, direct contact with animals and the environment. Although the serotype more frequently implicated in HUS is O157:H7, there are several other serotypes associated with human infections. Argentina has the highest incidence of HUS (17/100 000 children under 5 years old), and several serotypes have been isolated from human cases in this country: O157:H7, O145:H-, O178:H19, O91:H21 and serogroups as O26, O174, and O111. The aim of this work was to determine the serotypes shared among cattle, foods and environment and assess which are the most prevalent in a collection of VTEC isolated in Argentina.

**Material & Methods:** The isolates came from 4824 samples from cattle, foods (hamburger and minced meat) and environment of four different carcasses areas of 100 cm², and 65 g of beef products were processed according to the methodology described by USDA-FSIS 2008. STEC O157 strains were phenotypically and genotypically characterized. The subtyping by pulsed-field gel electrophoresis (PFGE) was performed using the 24-h PulseNet E. coli O157 standardized protocol.

**Results:** STEC O157:H7 strains were isolated from 3/60 (5%) ground beef and 1/7 (14.3%) fresh sausage samples, and characterized as stx2(c,8a-)/eae/ehxA (three strains), and stx2/eae/ehxA (one strain). Non-toxigenic E. coli strains were also isolated from 5 ground beef samples and one carcass, serotyped as O157:NT (five strains) and O157:NM (one strain). The strains yielded 10 different XbaI-PFGE patterns. By comparison with the Virulence profile database of STEC and E. coli O157 strains, 2149 strains/897 XbaI-PFGE patterns, none of the patterns have been previously detected in strains of different origins.

**Conclusions:** The most prevalent serotypes shared among cattle, foods and environment in Argentina have been also isolated from human cases in several countries including our country, and carry virulence profiles that reflect the pathogenic potential of the strains. These results reinforce the idea of using non-selective methods for the diagnosis of VTEC.

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**P-201**

**Characterisation of Sorbitol-Fermenting Escherichia coli O157 Strains from Red Deer**

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**Introduction & Objectives:** Sorbitol-fermenting (SF) Escherichia coli O157 strains have emerged as a cause of human diseases in continental Europe. Indeed, SF E. coli O157 accounts for 17% of sporadic cases of HUS and caused seven outbreaks in Germany between 1988 and 2009, and a large outbreak also occurred recently in the UK. In south-central Spain, large game hunting and game meat consumption are popular, and a major source of financial income. The aim of this work was to determine the phenotypic and genotypic features of four SF E. coli O157 strains isolated from hunter-harvested red deer in two different regions of Spain, with the objective of assessing the potential of deer as reservoir hosts of SF E. coli O157 strains pathogenic for humans.

**Material & Methods:** Strains were serotyped by standard procedures and tested for sorbitol fermentation, β-glucuronidase (GUD) activity and enterohaemolytic phenotype on sorbitol MacConkey (SMAC),
Chromocult Coliform and enterohaemolysin agars, respectively. Genotypic characterisation was performed by PCR and multilocus sequence typing (MLST).

**Results:** Serotype O157:H- was identified in the four strains. All of them fermented sorbitol and exhibited GUD activity after overnight incubation, and were non-phaemolytic. Since the strains were non-motile and their H antigen could not be determined by serotyping, the presence of the H7-encoding fliC gene was demonstrated in all the strains by PCR. All of them carried genes encoding the intimin subtype 7L, although no Shiga toxin-encoding genes (stx) were detected. Three strains carried genes encoding the enterohaemorrhagic *E. coli* (EHEC)-haemolysin and also the large plasmid that contains the EHEC-hlyA gene. All the strains carried the stp gene cluster and lacked the ter gene cluster, and were unable to grow on cefixime tellurite SMAC agar, which are typical features of SF *E. coli* O157:H- strains isolated from patients. In MLST analysis, three of the strains had identical alleles at all seven loci investigated and belonged to ST11, which comprises EHEC-hlyA and non-SF O157:H7/H- human isolates. The fourth strain differed from the former three in only one locus and belonged to ST587, which is a member of the ST11 complex and has also been identified among SF O157:H- human isolates.

**Conclusions:** These findings indicate that free-ranging red deer may be one of the possible reservoir hosts of SF *E. coli* O157 strains pathogenic for humans. Nevertheless, the demonstration of clonal relatedness between SF *E. coli* O157 strains isolated from deer and patients is required to provide evidence that deer can be a source of human diseases. In that respect, undercooked game meat and meat products obtained from deer and vegetables or water contaminated with deer faeces might be the most likely routes of transmission of SF *E. coli* O157 infection to humans.

**P-203**

**High Prevalence of *Escherichia coli* O157 Excretion on a Dairy Cattle Farm for Unpasteurised Milk Production**

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**Material & Methods:** Were investigated. In MLST analysis, three of the strains had identical alleles at all seven loci investigated and belonged to ST11, which comprises EHEC-hlyA and non-SF O157:H7/H- human isolates. The fourth strain differed from the former three in only one locus and belonged to ST587, which is a member of the ST11 complex and has also been identified among SF O157:H- human isolates.

**Conclusions:** These findings indicate that free-ranging red deer may be one of the possible reservoir hosts of SF *E. coli* O157 strains pathogenic for humans. Nevertheless, the demonstration of clonal relatedness between SF *E. coli* O157 strains isolated from deer and patients is required to provide evidence that deer can be a source of human diseases. In that respect, undercooked game meat and meat products obtained from deer and vegetables or water contaminated with deer faeces might be the most likely routes of transmission of SF *E. coli* O157 infection to humans.
the serotype O111:H8 harbored stx1, eae and hlyA genes; 4.6% were typed O26:H4 and had only the stx2 gene; finally, the rest of strains were O111 and some strains were positive for genes hlyA, stx1 and stx2 and others for hlyA, eae and stx2 genes. Several PFGE and MLVA profiles were obtained for the O157 STEC and non-O157 STEC, indicating genomic diversity among the 63 strains tested. 

**Conclusions:** The STEC presence in farm animal feces was confirmed, and provides novel information about STEC population in Northwestern region of Mexico. Further research might help to clarify the role of STEC in the environment and its pathogenicity for human.

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**Experimental Evaluation of the Basis of Seasonal Variation in Bovine Shedding of STEC O157**

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**Introduction & Objectives:** The major reservoir of STEC is healthy cattle and the frequency and numbers of STEC in feces of shedding animals varies strongly by season. The mechanism(s) underlying this variation is unknown. Higher summertime prevalence has been reported from diverse regions including the Netherlands, Italy, England, Canada, the United States, Turkey, and Korea. Seasonality of STEC shedding by cattle may be influenced by both exogenous factors such as ambient temperature-induced environmental proliferation or predation, and/or seasonal variation in feed components affecting STEC replication, as well as endogenous factors such as immune responses and possible endemic effects.

**Materials & Methods:** A 2 year study was undertaken to determine whether seasonal variation is due to factors intrinsic or extrinsic to cattle. Two cohorts of ten Holstein steers were housed in an open barn subject to seasonal temperature and humidity fluctuations. Feed was consistent for all seasons. Cattle were randomized into one of two groups by route of STEC oral or intra-rectal instillation and challenged in summer and winter. Each orally dosed animal received 10^7 cfu STEC O157 in saline and rectally dosed animals receive 10^7 cfu STEC O157 administered using a sponge. Inocula contained equal numbers of four strains belonging to the most common clinical- and bovine-biased genotypes: 1, 3, 5, and 6. Animals were sampled on days 1, 4, 7, and weekly thereafter for 60 days post-challenge and STEC measured by standard culture of the recto-anal junction mucosa. Presumptive STEC O57 were confirmed by O157-specific latex agglutination and genotypes were distinguished by PCR. Bedding, water and hair-coat were cultured weekly to determine the density of STEC O157.

**Results:** The results from the first year (two seasons) of this 2 year study will be presented.

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**Awareness and Management of E. coli O157 Risk Factors Remains Important for Private Farm Residents and Visitors, as well as for Open Farms**

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**Introduction & Objectives:** Scotland consistently reports higher rates of E. coli O157 infection than many other countries in Europe or North America, to which the ratio of human to cattle populations and reliance on private water supplies in farming areas may contribute. Approximately 20% of cases in Scotland are part of outbreaks. A large E. coli O157 outbreak at an open farm in England in 2009 demonstrated various public health dilemmas including reliance on voluntary controls and difficulties in preventing environmental exposures and cross-contamination. We reviewed data from enhanced surveillance in Scotland which suggests that not only open farm visitors, but also private farm residents and their visitors, are at risk.

**Materials & Methods:** We analysed data from Health Protection Scotland (HPS) surveillance systems for E. coli O157 covering (i) outbreaks, and (ii) all laboratory confirmed cases, for the period 1996–2010 (data for 2010 remain provisional).

**Results:** Provisionally, from 1 January 1996 to 31 December 2010, 23/128 (18%) E. coli O157 outbreaks involved visitors to, or residents or workers on, farm premises; animal or environmental exposures were the main reported modes of transmission.

Conclusions: Farm-related outbreaks accounted for almost a fifth of all E. coli O157 outbreaks in Scotland 1996–2010, and involved private rather than open farms. Whilst the average number of confirmed cases was slightly higher in open than private farm outbreaks, the total number affected in private farm outbreaks was more than twice that in open farm outbreaks, and children under 16 accounted for 75% of confirmed cases in both. The precautionary principle requires that all farms should be considered contaminated, and risk awareness is essential on all types of farm premises. Most official guidance for open farms (e.g. on separation of eating and livestock functions) is directed at open farms, but applies to all farms – especially the need for simple but essential precautions such as stringent hygiene. These data emphasise the importance of risk management on farms of all types, and the continuing need for agricultural, health and veterinary stakeholders to work in partnership.

**P-209**

**Phylogenetic and Molecular Characterization of Shiga Toxin-Producing Escherichia coli Isolated from Risk Food**

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**Introduction & Objectives:** In order to assess their virulence and risk potential, 75 food-associated Shiga toxin (stx)-producing Escherichia coli (STEC) isolates with an already known virulence gene spectrum, were investigated for their phylogenetic relatedness and occupation of pathogenicity island (PAI)- integration sites. Because important virulence determinants such as the locus of entero-cyto effacement (LEE) and genes for type III secretion systems were absent in nearly all of these strains, the question arose how far they pose a health risk for humans.
Materials & Methods: All strains were typed by multi locus sequence typing (MLST). Moreover, particular PAI integration sites were scanned for the insertion of foreign DNA by PCR. In addition, the presence of the PAI ICL3, the locus of proteolysis (LPA), and the sab gene was detected by PCR.

Results: MLST revealed 35 different profiles within the 75 analyzed strains. The sequence types of the strains correlated well with their serotypes and the most frequent clonal complex was CC155. Within this clonal complex, the most abundant serotype was O113:H21. Particular human strains were found to cluster together with some of the analyzed food isolates. The three LEE integrations sites selC, pheU and pheV were occupied in about 50% of the eae negative strains. Except in serotype O113:H21, a general correlation between MLST and the distribution of occupied integration sites could not be found. Phylogenetic analyses showed that the food isolates were distributed heterogeneously, and certain MLST clusters were not detected.

Conclusions: In conclusion, the results of this study demonstrated that no particular sequence type or clonal complex was associated with the analyzed food isolates. No differences between food and human sequence types from the same serotype were detected and the occupation of integration sites indicated additional genetic material of unknown origin. The identity of the foreign DNA has to be analyzed in further studies to better assess the risk potential of these strains.
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Effectiveness of Azithromycin and/or Chinese Medicine in the Early and Late Stage Infections of Shiga Toxin-Producing Escherichia coli-Infected Mouse Models
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Introduction & Objectives: We developed a fatal neurological mouse model by infecting with a high dose (10^11 CFU/mouse) of Shiga toxin 2c (Stx2c)-producing Escherichia coli O157:H- strain E32511/HSC (Streptomycin and Mitomycin resistant/SMM’ MMC”) in 1994 (Infect Immun 62:3447 3453). Furthermore, we reported that fosfomycin and kanamycin were not effective in preventing the fatality in this animal model (FEIMM Mol Med Microbiol. 26: 101–108, 1999). Recently, Zhang et al. (J Infect Dis. 199: 486–493, 2009) reported that pediatric doses of azithromycin (AZM), but not ciprofloxacin, was effective in treating a piglet model orally infected with Stx2c-producing E. coli. In our present study, we evaluated the effectiveness of AZM and/or Chinese medicine (herbal medicine, which is known to have a purgative effect in the intestinal tract) using our mouse model inoculated with 10^11 CFU/mouse of E32511/HSC (SM’ MMC”) and simultaneously injected intraperitoneally with MMC, which we presumed as a late stage of EHEC infection. We also tested the effectiveness of Chinese medicine alone by using the mouse model infected with Shiga toxin 2d-producing E. coli O91:H21 strain B2F1 (Streptomycin resistant/SM’) (Infect Immun. 61:3832–3842, 1993), which we presumed as an early stage of EHEC infection.

Material & Methods: ICR mice were infected with 10^11 CFU/mouse of E32511/HSC (SM’ MMC”) simultaneously injected intraperitoneally with MMC, thereafter treated with AZM 2, 6 or 24 h after inoculation and/or Chinese medicine 2 h after inoculation of E32511. Chinese medicine alone once per day, for five consecutive days, was given one day after oral inoculation of mouse with B2F1 (SM’) 10^8 CFU. Survival was observed for 2 weeks, and the data were statistically analyzed. Immunohistochemistry and pathological examinations were performed to observe the damages to mouse organs especially the brain.

Results: Based on the survival rate analysis, we found that the administration of AZM in a single dose was significantly effective when given 2 h after infection compared to 6 and 24 h after infection. Moreover, administration of AZM 6 h after infection combined with Chinese medicine 2 h after infection was also effective in this mouse model inoculated with 10^11 CFU of E32511/HSC (SM’ MMC”). In the mouse model inoculated with 10^8 CFU of B2F1(SM’), treatment with Chinese medicine alone, once a day for 5 days, a day after bacterial inoculation, was significantly effective in the mouse survival and protected the colon and small intestine from bacterial adhesion.

Conclusions: We found the effectiveness of AZM and its combination with Chinese medicine in our mouse model inoculated with E32511/HSC (SM’ MMC”). Moreover, only Chinese medicine was found to be effective in the mouse model inoculated with B2F1 (SM’).

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Significantly associated with death. Renal failure was more frequent among women than among men (age-adjusted OR was 4.06, \( P = 0.046 \)) during the two outbreaks in Kagawa and Tochigi prefectures. In the age-category of adults more than 20 years old, female was significantly more at risk of developing HUS in Japan (\( P < 0.001 \)).

**Conclusions:** The factors that are significantly associated with increased risk of death are as follows: bloody diarrhea, completely bedridden status, and WBC count in the home for the aged. We concluded that female was significantly more at risk of developing HUS in Japan.

**P-216**

**Antagonistic Properties of Saccharomyces cerevisiae CNCM I-3856 Against Enterohemorrhagic Escherichia coli O157:H7**

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**Introduction & Objectives:** Enterohemorrhagic Escherichia coli (EHEC) are important food-borne pathogens responsible for diarrhea, hemorrhagic colitis and life-threatening complications such as hemolytic-uremic syndrome (HUS). Among EHEC strains, O157:H7 is the main serotype involved in sporadic cases and outbreaks. Human contamination mainly occurs following ingestion of undercooked ground beef. Both the survival of EHEC strains in the human digestive environment and their interaction with the intestinal barrier (especially with M cells) are key factors in bacterial pathogenesis. Nevertheless, the related mechanisms remain unclear owing to lack of relevant models. As no specific treatment is available for EHEC infections and as antibiotic therapy has worsened clinical outcomes, alternative strategies using probiotics have been considered. Two complementary *in vitro* approaches have been used to better understand the behavior of EHEC in the human digestive environment and investigate the antagonistic properties of a new probiotic yeast strain, *Saccharomyces cerevisiae* CNCM I-3856.

**Material & Methods:** EHEC survival was determined during *in vitro* digestions of a standard western meal containing ground beef, inoculated with EHEC O157:H7 or EHEC with *S. cerevisiae*. The experiments were performed in the dynamic TNO gastro-Intestinal tract Model (TIM). Using an *in vitro* M co-culture model (Caco-2 and Raji B cells), we also investigated the ability of the probiotic yeast to prevent EHEC translocation.

**Results:** Bacterial mortality was observed in the stomach and duodenum of the TIM, showing the sensitivity of EHEC O157:H7 to gastric acidity and digestive secretions. By contrast, growth resumption was shown in the distal parts of the small intestine. The co-administration of *S. cerevisiae* CNCM I-3856 led to a significant decrease in bacterial growth, maybe through ethanol production. The probiotic yeast also showed antagonistic properties against EHEC by significantly decreasing the number of translocated bacteria across the *in vitro* M cell model.

**Conclusions:** Our study shows that *S. cerevisiae* CNCM I-3856 could be used both to reduce the amount of pathogenic bacteria reaching the large intestine and their uptake by M cells. This yeast emerges as a relevant probiotic agent that may be used in the fight against EHEC infections.

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**Peptide-Based Stx-Neutralizers for Treatment of STEC Infections**

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**Introduction & Objectives:** Shiga toxin (Stx) is a major virulence factor of Stx-producing *E. coli* (STEC), such as *E. coli* O157:H7, whose infection causes bloody diarrhea and hemorrhagic colitis in humans, sometimes resulting in fatal systemic complications. Stx binds to the cell-surface receptor, globotriaosyl ceramide (Gb3), through its B-subunit pentamer. Highly selective and potent binding of Stx to Gb3 is attributed to the multivalent interaction of the B-subunit pentamer with the trisaccharide moiety of Gb3. Stx-neutralizers that effectively inhibit the interaction and then its cytotoxicity can be potential therapeutic agents against STEC infections. Recently we developed a multivalent peptide-library technique and identified a tetravalent peptide (referred to as PPP-tet) that potently binds to and neutralizes Stx2, one of the known Stx family members. PPP-tet protected mice from challenge with a fatal dose of STEC. In this study, we applied this technique to identify a novel peptide-based neutralizer against Stx1, another major Stx family member.

**Material & Methods:** A tetravalent peptide-library was screened to determine a peptide motif that specifically binds to one of the three receptor-binding sites on Stx1 B-subunit, i.e. site 1. Using the identified peptide motifs, tetravalent peptides were synthesized and examined for their inhibitory activities *in vitro* and *in vivo*.

**Results:** We could identify four peptide motifs, and synthesized four tetravalent peptides with each motif. These tetravalent peptides specifically bound to the site 1 of Stx1 B-subunit, and efficiently inhibited the cytotoxicity of Stx1. Interestingly, one of the tetravalent peptides, MMA-tet, inhibited the cytotoxicity of Stx2 as well, even with more efficacy than that of PPP-tet. Furthermore, MMA-tet completely protected mice from a fatal dose of an *E. coli* O157:H7 strain that produces both toxins, when orally administered after an established infection.

**Conclusions:** The multivalent peptide-library technique can identify a series of peptide-based Stx-neutralizers with remarkable potency as promising therapeutic agents for treatment of STEC infections.
Introduction & Objectives: The clinical course of post-diarrheal HUS (D+HUS) can be unpredictable. Early knowledge of which children are most likely to have poor outcomes can improve clinical decision making. We assessed laboratory and clinical predictors of poor outcome in children with D+HUS using a classification tree-based modeling approach.

Material & Methods: FoodNet conducts active surveillance for HUS in children <18 years old in selected US states. We define D+HUS as acute anemia, azotemia, and thrombocytopenia diagnosed as HUS <3 weeks after diarrhea onset. Laboratory and clinical data were collected using standardized report forms. Laboratory results recorded were the highest [e.g., creatinine and white blood cell count (WBC)] or lowest (e.g., hematocrit and platelet count) values observed from 7 days before to 3 days after HUS diagnosis. We defined poor outcome as death during hospitalization, hospitalization duration >32 days (90th percentile), or ongoing dialysis or neurological deficit at discharge. Missing data among potential predictor variables were imputed using Random Forests (R randomForest package). Classification trees were built in JMP 9.0 software (SAS Institute Inc.) using the complete dataset of cases occurring during 2004–2010. A saturated tree was pruned to include only clinically relevant variables.

Results: Of the 401 D+HUS cases identified, 68 (17%) had poor outcome; 31 (8%) of 399 with available data were hospitalized >32 days, 23 (6%) of 369 required dialysis at discharge, 10 (2.7%) of 370 had neurological deficits at discharge, and 10 (2.5%) of 399 died while hospitalized. We observed no significant change over time in the proportion with poor outcome. Significant predictors of poor outcome were: (i) lowest hematocrit less than normal but ≥20%, (ii) highest WBC ≥24 000/μL, (iii) antibiotic treatment for reasons other than diarrhea in the 3 weeks before HUS diagnosis, and (iv) antibiotic treatment of the diarrheal illness. Children with the highest probability (88%) of poor outcome had hematocrit ≥20% and received antibiotics for both diarrheal and non-diarrheal illnesses. Children with the lowest probability (3%) had hematocrit <20% and WBC <24 000/μL. The tree had a sensitivity of 19% and a specificity of 99.2%. Other than diarrhea, respiratory and urinary infections were the most common reasons for taking antibiotics before HUS diagnosis.

Conclusions: Like others, we identified leukocytosis and mild (i.e., not severe) anemia as predictors of poor outcome. Our data indicate that recent antibiotic treatment for non-diarrheal illness or prodromal diarrhea may be important predictors. Antibiotics might influence severity through alterations of the intestinal microbiome favoring growth of Shiga toxin-producing organisms or through increased release of Shiga toxin. Because of its high specificity, our marker is a highly immunogenic protein with adjuvant properties. Antigenic epitopes in the stx2b coding sequence were cloned upstream to the bacterial carrier coding sequence (previously cloned in pET11a vector) using a 10 aminocacid linker. This construct was used for expression and purification of the recombinant protein (rChimera). The chimera coding sequence was also sub-cloned into pC1-neo plasmid (pChimera) for DNA vaccination. BALB/c mice were immunized three times with rChimera (20 μg Stx2B/dose) formulated in: (A) Freund’s Adjuvant (FA) (i.p.), (B) Alum Hydroxide (subcutaneous) and (C) without adjuvant (i.p.). In addition, a prime boost protocol (D) was evaluated, with three doses of pChimera (100 μg/dose, intramuscular) and one dose of rChimera in FA (i.p.). Mice were immunized with recombinant Stx2B in FA (E) (i.p.) as control.

Results: The Stx2B-specific IgG titer were determined by ELISA 45 days after the last immunization (n, Mean ± SEM): (A) 6, 124 890 ± 24 289; (B) 4, 7285 ± 2400; (C) 4, 688 ± 150; (D) 4, 6910 ± 2966; (E) 4, 924 ± 676. (*P < 0.05 versus B, C, D and E). In addition, the neutralizing activity was evaluated in vitro on Vero cells and the neutralizing titers (serum dilution that blocked a 50% cytotoxic dose of Stx2) were determined: (A) 1508 ± 335; (B) 178 ± 86; (C) 104 ± 66; (D) 202 ± 92; (E) 60 ± 48 (*P < 0.05 versus B, C, D and E).

To further analyze the protective capacity, one 100% lethal dose (1LD100) of Stx2 was pre-incubated with sera from experimental groups and injected intravenously (i.v.) into naïve mice. All mice given Stx2 pre-incubated with sera from non-immunized (serum dilution, n) (1/50, 11) or rStx2B (1/50, 5) mice, died within 96 h post-inoculation. In contrast, 100% survival was obtained when Stx2 was pre-incubated with sera from Chimera-immunized mice in all protocols: (A) 1/2000, 3*; (B) 1/50, 3*; (C) 1/50, 3*; (D) 1/100, 4* (*P < 0.05 versus non-immunized and rStx2B).

Finally, immunized and non-immunized mice were challenged with 1LD100 of Stx2. Although all non-immunized mice died within 96 h post-inoculation and only 33% of rStx2B mice survived, all Chimera-immunized mice in all protocols survived the challenge (*P < 0.05 versus non-immunized and rStx2B).

Conclusions: The high antibody and neutralizing titers and the complete protection obtained by immunization with the Chimera, suggests that this novel immunogen is a promising candidate for an HUS vaccine development or for the generation of therapeutic antibodies to be administered after STEC infection.

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ZAK: A Novel Therapeutic Target for Treating Shiga Toxin and Ricin Mediated Disease?
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Introduction & Objectives: Shiga toxins and ricin damage the 28S ribosomal subunit by exerting an A-subunit dependent n-glycosidase activity that results in the depurination of a single adenine residue from the evolutionarily conserved s-sarcin loop. The immediate result of this ribosomal insult is a halt in protein synthesis. In addition to inhibition of protein synthesis, this damage to the 28S ribosomal subunit results in the initiation of the ribotoxic stress response which entails activation of the MAP3Kinase signaling cascade and contributes to pro-inflammatory and pro-apoptotic events. Previous studies by our group have demonstrated in vitro that the MAPK3Kinase ZAK transduces the ribotoxic stress response by Shiga toxins and ricin, and that treatment of cells with a ZAK specific...
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inhibitor reduces IL-8 production and caspase-3 cleavage. Therefore we chose to further investigate whether ZAK plays a role in Shiga toxin and ricin induced pathogenesis using rabbit and mouse models respectively.

Material & Methods: For these studies we employed ZAK knockout mice and infant New Zealand white rabbits. *In vitro* ricin studies were performed on bone marrow derived macrophages (BMDMs) from ZAK knockout or wild type mice. ZAK knockout or wild type mice were also used for the *in vivo* oral ricin intoxication study. Infant rabbits were pre-treated with either the Bcr-Abl inhibitor imatinib or vehicle prior to administration of Shiga toxin. Western blot and real-time PCR were used to detect MAP Kinase activation and cytokine production respectively. All histological analyses were performed by blinded scoring of H&E stained tissue sections.

Results: Ricin treatment of bone marrow derived macrophages (BMDM) from ZAK knockout mice failed to induce p38 and JNKs phosphorylation compared with BMDMs from wild type mice. BMDMs from ZAK knockout mice also had decreased ricin induced transcription of IL-1β, TNF-α, CXCL-1, and CCL2. Oral administration of ricin to homozous ZAK knockout mice resulted in significantly less intestinal damage as compared to that of homozygous wild type littermates. Finally, oral pretreatment of infant rabbits with imatinib, a chemotherapeutic agent with affinity for ZAK resulted in a significant decrease in Shiga toxin induced heterophil infiltration of colonic tissue.

Conclusions: Together these data suggest that ZAK acts in vivo to transduce the ribotoxic stress response to Shiga toxins and ricin, and therefore ZAK may have potential for use as a therapeutic target for treating illnesses associated with these select agent toxins.

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Characterization of Four T1-like Lytic Bacteriophages that Lyse Shiga-Toxin Producing *Escherichia coli* O157:H7

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Introduction & Objectives: A previous field trial indicated that naturally-occurring bacteriophages (phages) were associated with reduced fecal shedding of Shiga-toxin producing *Escherichia coli* O157:H7 (EC O157) in cattle. This study aimed to characterize four endogenous phages with potential for controlling EC O157 in cattle.

Material & Methods: Common phage types of EC O157 strains (*n* = 14) isolated in Alberta, Canada and non-EC O157 strains (*n* = 2) were selected to assess their susceptibility to isolated phage. Four phages were serially diluted and incubated for 5 h with overnight bacterial cultures to estimate their multiplicity of infection (MOI). Purified phage particles were deposited on copper grids with carbon-coated Formvar films, stained with 2% K phosphoagulate (pH 7.2) and 2% uranyl acetate (pH 4.5), and examined in a Philips EM 300 electron microscope. Pulsed field gel electrophoresis was used to estimate genome size and restriction enzyme pattern of the four phages.

Results: All phages had plaques of 1–2 mm in diameter surrounded by translucent halos, when plated with modified nutrient agar overlaid with EC O157 strain R508 (Phage type 14, PT14). All 14 EC O157 strains were sensitive to phages vB_EcoS_AHP24 (AHP24) and vB_EcoS_AHS24 (AHS24), while only strains EC19990295 (PT4) and EC19990300 (PT2) were resistant to vB_EcoS_AHP42 (AHP42) and vB_EcoS_AKS96 (AKS96). None of the four phages lysed the two non-EC O157 strains. All four phages showed high virulence (Avg. MOI = 0.0003–0.0007, *P* > 0.2) to EC O157 strains tested excluding EC19990295, EC19990300 and E32511. The phage genome size ranged from 43.05 to 46.07 kb. Transmission electron microscopy revealed that all phages had isosahedral heads of 58 nm with tapered and noncontractile tail of 167 × 8–9 nm. Tails had cross-saturations with 5 nm periodicity and ended in at least one fibre about 30 nm in length. These findings suggest that these phages are likely to fall into the T1 group of the family Siphoviridae. However, genotypes of the four phages differed as determined by *EcoRI*- or *HindIII*-digestion profiles. Phages AHP24 and AHS24, isolated simultaneously from fecal pats and manure slurry from the same feedlot pen, exhibited the greatest identity among the four phages.

Conclusions: This study suggests that T1-like phages may have utility in bio-control of EC O157 in cattle and exhibit differences in lytic capability and host range. Further studies are required to evaluate these T1-like phages for reliable biocontrol of EC O157 in cattle.

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Shigatoxin Expression Inhibition by Anti-Inflammatory and Analgesic Compounds in Shigatoxin Producing *Escherichia coli*

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Introduction & Objectives: Enterohemorrhagic *Escherichia coli* (EHEC) is a food-borne pathogen causing hemorrhagic colitis and hemolytic-uremic syndrome, especially in children. Shiga toxin (Stx) is the major virulence factor of EHEC and is responsible for the more severe symptoms of the infection. EHEC can produce one or both of two antigenically distinct forms of Stx, Stx1 and Stx2 but epidemiological studies have revealed that Stx2 is the most important virulence factor associated with severe human disease. In this study, we examined the influence of ibuprofen and paracetamol on Stx2 synthesis and the ability of this compounds to inhibit Stx expression in EDL933 EHEC strain.

Material & Methods: The inhibitory concentrations of the anti-inflammatory and analgesic compounds were determined after 24 h of incubation using viable counting to detect bacterial growth. The bacteria were then incubated at different exposure periods, with ibuprofen and paracetamol at 0.25 X MIC of these compounds. Strains were grown to a final optical density at 600 nm (OD600) of 1 in LB broth. Shiga toxin protein was measured from bacterial culture supernatants by enzyme immunoassorption assay (ELA) using RIDA-SCREEN® Verotoxin (R-Biopharm Latinoamérica S.A.). Then, we proceeded with the RNA extraction using the commercial equipment NucleoSpin RNA II according to manufacturer’s specifications. The analysis of RNA expression was performed by reverse transcription and quantitative real time (RT PCRq) in order to amplify stx2 gene and the normalizing gene gapdh in the Rotor Gene equipment (Qiagen).

Results: The effect of this compounds on Stx expression, as assessed by ELA performed on culture supernatants, showed that ibuprofen and paracetamol inhibited basal toxin expression in strain EDL933 by 90%. Crossing points (CT) values were normalized to levels of gapdh mRNA. The 2−CT values was over 3 for the not induced strain and the 2−ΔCT values was below 0.5 for the induced ones.

Conclusions: Ibuprofen and paracetamol inhibited the Stx2 expression, and their effects were measurable at the level of toxin protein as well as RNA expression in EDL933 strain. We concluded that a significant decrease in stx2 mRNA level and Stx2 concentration was observed in conditioned media after 72 h of exposure.
A study of Social and Political History of Hemolytic Uremic Syndrome

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Introduction & Objectives: An analysis on how HUS became a public health issue since its discovery in the mid-60 until 2009 when the Ministry of Health approved a national program for control and prevention of the disease. The paper aims to describe the social history of the disease and the public health policies the State has been implemented to reduce the incidence of HUS. By analyzing of such history is that we study HUS as a scientific problem, a social problem and a political one. The results of this research intended to contribute to the knowledge of social and political determinants of the disease and to help decision markers.

Material & Methods: The data collection was conducted through an articulation of qualitative methodologies which included: secondary sources as a variety of documentation to be reviewed, primary sources as semi-structured interviews, and direct observation of the discussions around the proposed intervention of control and prevention of disease.

Results: The construction of a timeline allowed us to set the following main moments of the experience with the disease. The periodization was organized in three phases: discovery of the disease, investigation and first responders, which provides an overview of the development in biomedical knowledge and the emergence of the issue in both social and political problem. Different control and prevention policies to reduce the incidence of the disease that have been implemented since 2000 are described and analyzed. Finally, based on an explanatory model, indicators are built to identify the need of a public policy, the debate of different proposals, and the materialization of the National Program of HUS (with no effective implementation at the moment).

Conclusions: The analysis of the social history of the disease, the implemented policies and the national program of HUS, provides a comprehensive account of the development of biomedical research, their struggle to incorporate the issue into the health agenda, and the resolution of some of its aspects and the challenges that still remain outstanding accounts by the health authority.
teins. Figure A gives a detailed view of the ME0052 (orange stick) binding mode to oxidised Tpx. A hydrogen bond (yellow line) is formed between the inhibitor and the carbonyl of I153. Figure B shows co-crystalisation of ME0052 (blue) with WrbA (subunits shown in purple and cyan) where the enzyme cofactor FMN and a tryptophan from an adjacent subunit have been highlighted in grey.

**Conclusions:** Evolution of the current SA is now possible using in silico drug design with the aim to improve the specificity and efficacy of this novel class of anti-virulence compounds.