Zoonoses
AND PUBLIC HEALTH

EDITOR: MARY TORRENCE

Abstracts from the 8th International Symposium on Shiga Toxin
(Verocytotoxin) Producing Escherichia coli Infections

6–9 May 2012
Amsterdam, The Netherlands

Disclaimer

This abstract book has been produced using author-supplied copy. Editing has been restricted to corrections of spelling and style where appropriate. No responsibility is assumed for any claims, instructions, methods or drug dosages contained in the abstracts; it is recommended that these are verified independently.
Editorial

In 1983, Mohamed Karmali et al. at the Hospital for Sick Children in Toronto reported an association between the haemolytic uraemic syndrome of childhood and the presence of a cytotoxin (termed verocytotoxins in that communication) in the stools of these patients. They identified *Escherichia coli* of various serotypes, including but not limited to those expressing O157 and H7 antigens, as the source of these toxins (Karmali et al., 1983). One week later, Riley et al., (1983) more formally introduced *E. coli* O157:H7 to the world as a cause of bloody diarrhoea (‘haemorrhagic colitis’), which was associated with the consumption of poorly cooked hamburgers. Several months later, O’Brien et al., (1983) reported that *E. coli* O157:H7 made a toxin that was quite similar to that made by *Shigella dysenteriae* serotype 1. These three cornerstone papers spawned a generation of science in the fields of gastrointestinal biology, veterinary microbiology and epidemiology, vascular pathology, nephrology, diagnostic microbiology, evolutionary biology, microbial pathogenesis, food safety and immunology.

Barely 4 years later, Dr Karmali, assisted by Drs Peter Fleming, Martin Petric and James Brunton, convened the First International Symposium on Shiga Toxin (Verocytotoxin) Producing *Escherichia coli* Infections (the ‘VTEC Meeting,’ to those in the field) in Toronto. There were only 90 registrants, but their areas of expertise presaged the ecumenical science that continues to characterize research on these toxin-producing pathogens. Speakers represented molecular, evolutionary, diagnostic and food microbiology. Clinicians included paediatricians and internists, subspecialists in gastroenterology, nephrology, infectious diseases and intensive care. Veterinarians, epidemiologists, cell biologists, and protein and lipid chemists presented data. The meeting was a standout in our memory, especially for those of us who were just launching our careers.

The VTEC Meeting has convened triennially since 1994 and rotated between continents (Bergamo, Italy (1994); Baltimore, USA (1997); Kyoto, Japan (2000); Edinburgh, Scotland (2003); Melbourne, Australia (2006); Buenos Aires, Argentina (2009); and Amsterdam, Netherlands (2012)). Each meeting reported the tremendous growth of the field and exemplified inclusive and collaborative scientific inquiry. Research centring around a group of bacteria that reside in colons of cattle, and which contaminate many different kinds of food, infect humans of all ages and in many different countries, and then elaborate a toxin that damages blood vessels, produce thrombi, and injure the kidneys and the brain, is highly likely to produce eclectic and lively meetings. However, the success of these gatherings is equally attributable to the skills and efforts of the local scientific hosts and organizing committees, who have perpetuated the intellectual and collegial spirit of the first Toronto meeting. Each Symposium has been an increasingly ‘tough act to follow,’ but each meeting has risen to the challenge.

In May of this year, Drs Nicole van de Kar and Reinhard Würzner continued the VTEC Meeting tradition by hosting the immensely successful Eighth International Symposium on Shiga Toxin (Verocytotoxin) Producing *Escherichia coli* Infections in Amsterdam. There were 540 attendees from 41 countries. The data, coming in the aftermath of the tragic *E. coli* O104:H4 outbreak in Germany last year, were voluminous and provocative, and the interchanges were friendly and robust. The venue, ambiance and data were outstanding.

In this issue, the VTEC Meeting now shares with the world a glimpse of the fine work presented. Specifically, the local committee chose to provide the meeting abstracts, so that interested parties who did not attend can glimpse the science that will appear in print in the coming months and years. Publishing these abstracts is voluntary and should not be considered to be definitive or of sufficient depth to preclude more detailed communications in peer reviewed journals. *Zoonoses and Public Health*, a journal dedicated to the interface between human health, veterinary biology and disease control, is delighted to provide this resource.

Finally, we congratulate Drs Todd Callaway, Lawrence Goodridge, and Jeff L'Jeune on their successful bid to host the ninth VTEC meeting in Boston in 2015. They have eight tough acts to follow.

Phillip I. Tarr
Associate Editor, Zoonoses and Public Health
Mary Torrence
Editor, Zoonoses and Public Health

References


**Poster Presentations**

**Prevention, Control and Treatment: Animal and Human**

**P-213**

**Effectiveness of Azithromycin and/or Chinese Medicine in the Early and Late Stage Infections of Shiga Toxin-Producing Escherichia coli-Infected Mouse Models**

M. Y. Amran¹, J. Fuji¹ and S. Yoshida²

¹Department of Neurology, Hasanuddin University, Makassar, Indonesia; ²Kyushu University, Department of Bacteriology, Fukuoka, Japan

**Introduction & Objectives:** We developed a fatal neurological mouse model by infecting with a high dose (10⁹ CFU/mouse) of Shiga toxin 2c (Stx2c)-producing E. coli O157:H- strain E32511/HSC (Streptococmycin and Mitomycin resistant/SM⁺ 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 (FEMS Immunol 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 10¹¹ 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 (Streptococmycin 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 10¹¹ 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⁻) 10⁸ 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 10¹¹ CFU of E32511/HSC (SM⁺ MMC⁻). In the mouse model inoculated with 10⁹ 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⁻).

© 2012 The Authors
Zoonoses and Public Health © 2012 Blackwell Verlag GmbH

**P-214**

**Risk Factors for Hemolytic Uremic Syndrome and Death caused by Shiga Toxin-Producing Escherichia coli Infection in Japan**

J. Fuji¹, T. Mizoue², Y. Nakada³, S. Ugajin⁴, Y. Naya⁴, T. Nakamura⁵, K. Saitoh⁶, Y. Maruyama⁶, Y. Tada⁷, N. Okabe⁷ and S. Yoshida¹

¹Department of Bacteriology, Kyushu University, Fukuoka, Japan; ²International Medical Center of Japan, Department of Epidemiology and International Health, Tokyo, Japan; ³Utsunomiya City Institute of Public Health and Environment, Department of Epidemiology and International Health, Utsunomiya, Japan; ⁴Utsunomiya City Public Health Office, Department of Epidemiology and International Health, Utsunomiya, Japan; ⁵Kagawa Prefectural Shozu Regional Health and Welfare Office, Department of Epidemiology and International Health, Tonoshocho, Shozugun, Japan; ⁶Kagawa Prefectural Chusan Regional Health and Welfare Office, Department of Epidemiology and International Health, Marugame, Japan; ⁷National Institute of Infectious Diseases, Infectious Disease Surveillance Center, Tokyo, Japan

**Introduction & Objectives:** As of 2011, a total of 4075 cases infected by Shiga-toxin2-producing enterohaggregative E. coli O104:H4, serogroup O104 have been reported in Germany. Most of the patients who developed hemolytic uremic syndrome (HUS) were adults and women were overrepresented (68%). In 2002, an outbreak of E. coli O157:H7 infection occurred among patients of psychiatric inpatient unit and residents of homes for the aged in Tochigi prefecture, Utsunomiya City, Japan. One hundred twenty-three developed diarrhea or bloody diarrhea, five developed HUS, and 10 patients died during this outbreak. In 2005, two clusters of diarrhea cases occurred among patients of two homes for the aged in Kagawa prefecture, Japan. Of the 263 elderly residents, 39 developed gastrointestinal illness including bloody diarrhea, five developed HUS, and seven patients died. Based on the clinical signs and laboratory data, we report useful predictive clinical risk-factors.

**Material & Methods:** Demographic characteristics including age and sex, presence or absence bloody diarrhea, detection of E. coli O157 in stool specimens, bedridden status, laboratory variables and antibiotic therapy were assessed in relation to outcome measures of death by statistical analysis. Statistical significance for the difference in frequency between categorical variables was assessed by Fisher’s two-tailed exact test. The difference in outcome measures (renal failure including HUS) between females and males was statistically tested by means of the Mantel-Haenszel method to adjust for age classes. Finally, we performed epidemiologic study related sex distribution of HUS patients infected with Shiga-toxin-producing E. coli from 2006 to 2009 in Japan. The number of men and women and proportion of women who developed HUS were calculated by three age-categories. One-sample test of proportion was used to test the difference of sex for each combination of age-category.

**Results:** In the outbreak in Kagawa prefecture, bloody diarrhea was significantly associated with death. And also completely bedridden status was significantly associated with death (adjusted OR, 7.2; 95% CI, 1.32–39.). In a univariate analysis of laboratory investigations, white blood cell (WBC) count >10.2 × 10⁵ cells/ml (P = 0.039) was
Introduction & Objectives: Enterohemorrhagic Escherichia coli O157:H7 is a relevant probiotic agent that may be used in the fight against the human digestive environment and their interaction with the intestinal barrier (especially with M cells) are key factors in bacterial growth, maybe through ethanol production. The probiotic yeast also showed antagonistic properties against EHEC by significantly associated with death. Renal failure was more frequent among women (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

L. Etienne-Mesmin1, V. Livrelli2, B. Chassaing2, J. Thévenot1, S. Denis3, S. Chalançon3, M. Alric4 and S. Blanquet-Diot4
1Université D'Auvergne, JE 2526 USC INRA 2018 and ERT CIDAM, Clermont-Ferrand, France; 2Université D'Auvergne, IE 2526 USC INRA 2018, Clermont-Ferrand, France; 3Université D'Auvergne, ERT CIDAM, Clermont-Ferrand, France.

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 cell 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.

P-218 Peptide-Based Stx-Neutralizers for Treatment of STEC Infections

M. Watanabe-Takahashi1, K. Tsutsuki2, E. Kita2 and K. Nishikawa1
1Doshisha University, Faculty of Life and Medical Sciences, Kyoto, Japan; 2Nara Medical University, Department of Bacteriology, Nara, Japan

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 diarrheas 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.

P-220 Predictors of Poor Outcome in Children with Post-Diarrheal HUS – United States, 2004–2010

1Centers For Disease Control and Prevention, Division of Foodborne, Waterborne, and Environmental Diseases, Atlanta, USA; 2Connecticut Emerging Infections Program, Yale University, New Haven, USA; 3Colorado State Government, Department of Public Health and Environment, Denver, USA; 4Georgia State Government, Department of Human Resources, Atlanta, USA; 5Maryland State Government, Department of Health and Mental Hygiene, Baltimore, USA; 6Minnesota State Government, Department of Health, Saint Paul, USA; 7The University of New Mexico, The New Mexico Emerging Infections Program, Albuquerque, USA; 8New York State Government, Department of Health, Rochester, USA; 9Oregon State Government, Public Health Division, Portland, USA; 10Tennessee State Government, Department of Health, Nashville, USA
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 >32 days (90th percentile), or ongoing dialysis or neurological deficit at discharge. Missing data among potential predictor variables produced an estimated overall accuracy of 94% and a sensitivity of 95% for poor outcome.

Results: Of the 401 D’HUS cases identified, 68 (17%) had poor outcomes; 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 95% 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 tree may be useful as a clinical algorithm to identify children at low risk for poor outcome.

P-222
A Novel Stx2B-Based Immunogen is Able to Confer Total Protection Against Stx2 Challenge
M. P. Mejias1, G. Ghersi2, C. A. Panek1, R. J. Fernandez-Brando1, G. Cabrera1, V. Zylberman2, F. Goldbaum2 and M. S. Palermo1
1Instituto De Medicina Experimental (IMEX-CONICET), Academia Nacional De Medicina, División Inmunología, Ciudad Autónoma De Buenos Aires, Argentina, 2Fundación Instituto Leloir, Inmunova, Ciudad Autónoma De Buenos Aires, Argentina

Introduction & Objectives: Infection with Shiga Toxin II (Stx2) producing Escherichia coli (STEC) causes hemorrhagic colitis and can progress to the life-threatening complication known as Hemolytic Uremic Syndrome (HUS). The aim of this work was to generate and evaluate a novel immunogen against Stx2. For this purpose, the B subunit of Stx2 (Stx2B, binding subunit) was linked to a bacterial protein scaffold (the resulting protein was named Chimera). This carrier is a highly immunogenic protein with adjuvant properties.

Material & Methods: The stx2b coding sequence was cloned upstream to the bacterial carrier coding sequence (previously cloned in pET11a vector) using a 10 aminocid linker. This construct was used for expression and purification of the recombinant protein (rChimera). The chimera coding sequence was also sub-cloned into pcDNA 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) (intrapitoneal, 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 titers 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 naive 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.

P-223
ZAK: A Novel Therapeutic Target for Treating Shiga Toxin and Ricin Mediated Disease?
D. M. Jandhyala1, J. Wong2, S. E. Stone3, N. J. Mantis4, B. E. Magun2 and C. M. Thorpe1
1Tufts Medical Center, Department of Geographic Medicine and Infectious Disease, Boston, USA; 2Oregon Health and Science University, Department of Cell and Developmental Biology, Portland, USA; 3New York State Department of Health, Division of Infectious Disease, Wadsworth Center, Albany, USA

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 MAPKinase signaling cascade and contributes to pro-inflammatory and pro-apoptotic events. Previous studies by our group have demonstrated in vitro that the MAP3Kinase ZAK transduces the ribotoxic stress response by Shiga toxins and ricin, and that treatment of cells with a ZAK specific inhibitors could prevent cell death.
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 (BMDMs) 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 homoyzous ZAK knockout mice resulted in significantly less intestinal damage as compared to that of homoyzous 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.

**P-224**

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

Y. D. Niu1, K. Stanford2, H. W. Ackermann3 and T. A. McCallister1

1Lethbridge Research Centre, Agriculture and Agri-Food Canada, Department of Sustainable Production System, Lethbridge, Canada; 2Alberta Agriculture and Rural Development, Department of Livestock Research, Lethbridge, Canada; 3Department of Medical Biology, Laval University, Quebec, Canada

**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 serial 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 phosphohumate (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 on overnight Shiga-toxin producing cultures. The phage 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 isoschahedral heads of 58 nm with tapered and noncontractile tail of 167 × 8–9 nm. Tails had cross-striations 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.

**P-225**

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

C. Casabonne,1 I. Bolognino, V. Aquili, T. Subils and C. Balagüé

Biochemical and Pharmaceutical Sciences Faculty, Department of Bacteriology, Rosario, Argentina

**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 inhibition of Stx synthesis, and their effects were measurable at the level of toxin protein expression, and 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.

**Results:** The effect of this compounds on Stx expression, as assessed by ELISA performed on culture supernatants by enzyme immunoadsorption assay (ELISA) 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 PCR) in order to amplify stx2 gene and the normalizing gene gapdh in the Rotor Gene equipment (Qia-gen).

**Conclusions:** The effect of these compounds on Stx expression, as assessed by ELISA 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−Ct values was below 0.5 for the induced ones. 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

M. B. Belardo

Conicet, Dpto de Salud y Población, Instituto de Investigación Gino Germani, Facultad de Ciencias Sociales, Universidad de Buenos Aires, Buenos Aires, Argentina

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.

P-226

A study of Social and Political History of Hemolytic Uremic Syndrome

M. B. Belardo

Conicet, Dpto de Salud y Población, Instituto de Investigación Gino Germani, Facultad de Ciencias Sociales, Universidad de Buenos Aires, Buenos Aires, Argentina

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.

P-228

Lipopolysaccharide-Sensitized Transgenic Mice Constitutively Expressing Human Serum Amyloid P Component are Protected from Stx2 by Polymeric Heterobifunctional Ligands

J. M. Jacobson¹, P. I. Kitov¹, G. L. Mulvey², T. P. Griener², D. R. Bundle³ and G. D. Armstrong²

¹Department of Chemistry, University of Alberta, Edmonton, Canada; ²Department of Microbiol., Immunol., Infect. Dis., University of Calgary, Calgary, Canada

Introduction & Objectives: We previously reported that Pk-polyBAIT, a linear acrylamide polymer containing tethered heterobifunctional groups composed of the Shiga toxin 1 (Stx1) Pk-trisaccharide (Gb3) receptor and a human serum amyloid P component (HuSAP) ligand, 1,3-cyclic pyruvate ketal of glycerol, induced face-to-binding of the Stx1 B pentamer with HuSAP. As a consequence, Pk-polyBAIT effectively inhibited Stx1 from binding to Gb3 receptor sequences immobilized in 96 well microtiter plates or on Vero cells thereby preventing its cytotoxicity. Pk-polyBAIT also protected HuSAP transgenic mice from Stx1-mediated toxemia. However, because HuSAP alone neutralizes Stx2, we were unable to determine the in vivo efficacy of Pk-polyBAIT constructs in Stx2-challenged HuSAP transgenic mice. Since these earlier reports, however, we demonstrated that HuSAP transgenic mice are rendered susceptible to Stx2 if they also receive an injection of purified lipopolysaccharide (LPS).

Material & Methods: Groups (n = 10) of mice were I.P. injected with 225 pg/g body weight Synsorb-Pk-purified Stx2 (<0.001 EU/µg protein) combined with 300 ng/g body weight E. coli O55 LPS (Sigma) or 225 pg/g body weight Stx2 combined with 300 ng/g body weight LPS and then immediately injected (I.V.) with Pk- or PkNAc-polyBAIT. Animals were monitored every 2–4 h and euthanized by CO2 asphyxia if signs of toxemia (lethargy) became apparent.

Results: We have exploited the LPS-sensitized HuSAP transgenic mouse system to demonstrate that Pk-polyBAIT protects mice from Stx2. Moreover, a polyBAIT derivative containing the Pk trisaccharide in which the terminal galactose was replaced by N-acetylgalactosamine (PkNAc: α-GalNAc-(1 → 4)-β-Gal-(1 → 4)-β-Glc), as reported by Kale R. R., et al. (Angew. Chem, Int. Ed. 2008, 47:1265–68), was even more effective than the original Pk-polyBAIT at protecting LPS-sensitized HuSAP transgenic mice from Stx2.

Conclusions: These results suggest that PkNAc-polyBAIT might reduce the occurrence of the hemolytic-uremic syndrome (HUS) in children suffering from enterohemorrhagic E. coli-mediated hemorrhagic colitis.

P-230

Development of Novel Anti-Virulence Compounds that Repress Expression of the E. coli O157:H7 Type 3 Secretion System

K. S. H. Beckham¹, M. Gabrielsen¹, V. Feher², B. O. Smith³, R. Rommie², O. Byron⁴ and A. J. Andrew¹

¹Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, UK; ²Biomedical Sciences Program, University of California, California, USA; ³School of Life Sciences, University of Glasgow, Glasgow, UK

Introduction & Objectives: The salicylidene acylhydrazides (SA) are a class of compounds that down-regulate the expression of the Type 3 Secretion System in several Gram-negative pathogens. Unlike traditional bactericidal antibiotics, these compounds reduce virulence without killing the bacteria and therefore selection for resistance should be greatly reduced.

Material & Methods: In order to determine the mode of action of the SA, their target proteins were identified by an affinity pull-down assay. Structural characterisation of the target proteins using a combination of biophysical techniques including X-ray crystallography, NMR, small-angle X-ray scattering and analytical ultracentrifugation has enabled us to determine the discreet binding of the SA ME0052.

Results: Several putative binding proteins were identified. Amongst these proteins was thiol peroxidase (Tpx); a peroxiredoxin involved in oxidative stress recovery, and the tryptophan repressor binding protein (WrbA); a NAD(P)H/quinone oxidoreductase whose role has yet to be fully determined. In this work the high resolution structure of thiol peroxidase in combination with NMR chemical shift data and molecular modelling has revealed the likely binding site of ME0052 on Tpx (Figure A). Furthermore, co-crystallisation of WrbA with ME0052 has been achieved (Figure B). The figures show the binding of the SA compound ME0052 with target proteins.
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.