ECMIS

2-5 June 2019, Ghent, Belgium

Ghent University
Faculty of Veterinary Medicine
Laboratory of Immunology
Salisburylaan 133 - 9820 Merelbeke
Belgium

Contact:
Professor Dr. Eric Cox – Head of Laboratory of Immunology
Tel: 0032 (0) 9 264 73 96
E-mail: Eric.Cox@UGent.be
Secretary of Immunology – PA to Prof. Cox
Tel: 0032 (0) 9 264 73 97

ECMIS
2-5 June 2019, Ghent, Belgium

GlaxoSmithKline

The **Reference**
in **Prevention**
for **Animal Health**

ECMIS

2-5 June 2019, Ghent, Belgium

Welcome to the 3rd "*E. coli and the Mucosal Immune System*" Symposium

This is the 3rd ECMIS meeting which aims to bring together basic scientists and clinicians in the field of *Escherichia coli and the Mucosal Immune System.* Step by step science resolves the fascinating bidirectional communication of *E. coli* with their host, but also the important role of microbiota in steering this communication. The fast appearance of highly antibiotic resistant *E. coli* has become a treat and one of the important goals is to use these new insights to develop innovative intervention strategies. During this meeting some of the most recent strategies will be presented.

It is not only the duty of scientist to share their discoveries via articles with the scientific community but it is as important to present them to specialists and discuss them with young scientists. ECMIS 2019 has become a full of science sparkling program. Besides the invited presentations, there will be 14 selected oral presentations of which several by PhD students.

Ghent is a unique location. It is a beautiful historical Flemish city filled with curious tourists exploring all romantic spots and between all these tourist more than 60,000 enthusiastic students keep the city young and sparkling. We are quite sure that the science and the city of Ghent will make this event an unforgettable experience.

ECMIS
2-5 June 2019, Ghent, Belgium

TABLE OF CONTENTS

LOCAL ORGANISING COMMITTEE 5

SCIENTIFIC COMMITTEE 6

THE VENUES 7

SCIENTIFIC PROGRAM SUNDAY JUNE 2 8

SCIENTIFIC PROGRAM MONDAY JUNE 3 9

SCIENTIFIC PROGRAM TUESDAY JUNE 5 11

SCIENTIFIC PROGRAM WEDNESDAY JUNE 5 13

PART I: INVITED LECTURES 14

PART II: SELECTED ORAL PRESENTATIONS 29

PART III: SELECTED POSTER PRESENTATIONS 43

LIST OF ATTENDEES 59

LIST OF INVITED SPEAKERS 65

ECMIS
2-5 June 2019, Ghent, Belgium

LOCAL ORGANISING COMMITTEE

Eric Cox
Laboratory of Immunology, Faculty of Veterinary Medicine, UGent, Belgium
Eric.Cox@UGent.be

Bert Devriendt,
Laboratory of Immunology, Faculty of Veterinary Medicine, UGent, Belgium
B.Devriendt@UGent.be

Herman Favoreel,
Laboratory of Immunology, Faculty of Veterinary Medicine, UGent, Belgium
Herman.Favoreel@UGent.be

Daisy Vanrompay
Molecular Biotechnology, Faculty of Bioscience Engineering, UGent, Belgium
Daisy.Vanrompay@UGent.be

ECMIS
2-5 June 2019, Ghent, Belgium

SCIENTIFIC COMMITTEE

Frederic Auvray
Université Toulouse Paul Sabatier, France

Eric Cox
Laboraty of Immunology, Faculty of Veterinary Medicine, UGent, Belgium

Bert Devriendt
Laboraty of Immunology, Faculty of Veterinary Medicine, UGent, Belgium

Henri De Greve
VIB - Laboraty of Structural and Molecular Microbiology, VUB, Belgium

Edward Dudley
The Pennsylvania State University, Pennsylvania, US

James Fleckenstein
Washington University, Missouri, US

Bruno Goddeeris
Faculty of Bioscience Engineering, KULeuven, Belgium

Marc Heyndrickx
Technology and Food Science Unit, ILVO, Belgium

Jacques Mainil
Department of Infectious Diseases, Faculty of Veterinary Medicine, ULG, Belgium

Meryem Aloulou
Université Toulouse Paul Sabatier, France

Jan Paeshuyse
Laboratory for host pathogen interactions, KULeuven, Belgium

Weiping Zhang
University of Illinois, Illinois, US

ECMIS
2-5 June 2019, Ghent, Belgium

THE VENUES

The **scientific venue** for the symposium will be Het Pand.

Convention Centre Het Pand, a former Dominican monastery, is located in the historical centre of the city, on the banks of the river Leie. The oldest parts of this impressive building dates back to the 13th century. During five centuries it was extended and refashioned in different styles. Since its rehabilitation and restoration, completed in 1991, Het Pand has been offering a wide range of possibilities for the organisation of scientific congresses and cultural events.

Het Pand houses a number of values collections, such as the rich ethnographical and archaeological collections of the University, the Museum of history of Medicine, full-size photographs of paintings by Brueghel and Early Flemish Masters, and an interesting collection of stained glass.

Convention Centre Het Pand:
Onderbergen 1, 9000 Gent

The symposium **dinner** will take place at the the beautiful Thagaste Monastery in the historic heart of Ghent. Thagaste is set in the unique location of a historic monastery where Augustinian monks still live and work. The monastery hosts a range of large and small meeting rooms, remarkable art treasures, a beautiful inner courtyard, a historic library and a Baroque church with valuable organs.

Thagaste Monasterium:
Academiestraat1, 9000 Gent

www.ecmis.ugent.be

SCIENTIFIC PROGRAM SUNDAY JUNE 2

17:00-18:00 **Registration**

18:00-18:05 **Opening Introduction**

Keynote lectures

Chairman Bruno Goddeeris

18:05-18:50 "***Role of the maternal microbiota in shaping gene expression and microbiota composition in the offspring intestine"*** *by Stephanie Ganal-Vonarburg, Department for BioMedical Research, University of Bern, Switzerland*

18:50-19:30 "**Cathelicidins, host defense peptides against *E. coli* infection**" *by Henk Haagsman, University of Utrecht, The Netherlands*

19:30 **Reception**

ECMIS
2-5 June 2019, Ghent, Belgium

SCIENTIFIC PROGRAM MONDAY JUNE 3

Chairmen Meryem Aloulou and Eric Cox

Mucosal immune responses

9:00 **Keynote lecture: "*T-dependent humoral responses in mucosal sites*"** *by Aloulou Meryem, CRCN, INSERM, Center for Pathophysiology of Toulouse Purpan, Toulouse University, France*

Modulation of the host

9:40 **Keynote lecture**: **"New insights regarding the interplay between *Shigella* and human lymphocytes**" *by Katja Brunner, Institut Pasteur, France*

10:20-10.50 **Coffee break**

10:50 **Selected oral presentations**
10:50 "The effects of β-1,3-1,6 glucans on innate immune responses in pigs" *by Leen Hermans, Ghent University, Belgium*

11:10 "The pathogenic mechanisms of QS-1 in Avian Pathogenic *E. coli" by Guoqiang Zhu, Yangzhou University, China*

11:30 – 13:00 **Lunch and poster session**

Chairmen James Fleckenstein and Bert Devriendt

13:00 **Keynote lecture: "Remodeling of intestinal epithelial architecture by enterotoxigenic *E. coli*"** *by James Fleckenstein, Washington University School of Medicine, Missouri, USA.*

13:40 **Selected oral presentations**
13:40 "Porcine small intestinal enteroids as a model to explore host-pathogen interactions" *by Bjarn Vermeire, Ghent University, Belgium*

14:00 "*Escherichia coli* ST131: a versatile multidrug-resistant pathogen in and outside the gut" *by Makrina Totsika, Queensland University of Technology, Australia*

14:20 – 14:50 **Coffee break**

Chairmen Åsa Sjöling and Jacques Mainil

Modulation of *E. coli* by the host

14:50 **Keynote lecture: "*Bile salts and other host factors regulate expression of ETEC virulence genes*"** by *Åsa Sjöling, Department of Microbiology, Tumor and Cell Biology (MTC), Karolinska Institute, Sweden*

15:30 **Selected oral presentations**

15:30 "Oxygen and contact with human intestinal epithelium independently stimulate virulence gene expression in enteroaggregative *Escherichia coli" by Stephanie Schüller, University of University of East Anglia, UK.*

15:50 "Quorum sensing signal Acyl-Homoserine Lactones enhance the acid resistance of Enteropathogenic *Escherichia coli* O157:H7 by activating the rpoS and gad system" *by Guoqiang Zhu, Yangzhou University, China*

15:50 **Finish of day one**

17:30 **Guided tour in the Historical Centre of Ghent or Bruges (by bus)**

ECMIS
2-5 June 2019, Ghent, Belgium

SCIENTIFIC PROGRAM TUESDAY JUNE 5

Chairmen Edward Dudley and Marc Heyndrickx

Modulation of *E. coli* by the environment

9:00 **Keynote lecture: "Stx Phages and their induction"** *by Frederic Auvray, Digestive Health Research Institute (IRSD: UMR INSERM 1220, INRA 1416, ENVT, UT3), France*

9:40 **Keynote lecture: "Commensal *E. coli* that enhance toxin production by *E. coli* O157:H7"** *by Edward G. Dudley, The Pennsylvania State University, US*

10:20-10:50 **Coffee break**

10:50 **Selected oral presentations**
10:50 "Inactivation of stx-phages by probiotic *E. coli* strain Nissle 1917" *by Soundararajan M, Institute for Molecular Infection Biology, Wuerzburg, Germany*
11:10 "Vitamin K influences the virulence potential of Enterohemorrhagic *Escherichia coli" by Kijewski Anne, Norwegian Institute of Life Sciences, Norway*

11:30 – 12:30 **Lunch**

12:30 – 13:30 **Poster sessions – Walking tour**

Chairmen Christina Schäffer and Henri De Greve

Host-pathogen interaction at the receptor level

13:30 **Keynote lecture: "Possible roles of glycobiology for establishment and persistence of bacteria in the host***" by Christina Schäffer, BOKU University of Natural Resources and Life Sciences, Austria*

Preventive measures

14:10 **Keynote lecture**: **"Receptor analogues as strategy to prevent *E. coli* infection"** *by Eric Cox, Ghent University, Belgium*

14:50 -15:10 **Coffee break**

15:10 **Selected oral presentations**
15:10 "Human cellular and humoral immune responses against colonization factors and mucinase YghJ after experimental infection with an epidemiologically relevant STh-only ETEC strain" *by Hanevik Kurt, University of Bergen, Norway*

15:30 "Improved weight gain and reduced mortality following oral vaccination of pigs with Coliprotect® F4" *by Vangroenewhege Frédéric, Elanco Animal Health, Belgium*

15:50 “Antibodies derived from STatoxoid-mnLTR192G/L211A toxoid fusions induce neutralizing antibody against LT and STa but show little cross-reactivity with guanylin or uroguanylin” by Duan QQ, Yangzhou University, China

16:10 **Keynote lecture**: “**In feed administered non-encapsulated monomeric porcine IgA antibodies produced in yeast as prophylaxis against F4-mediated colibacillosis in pigs”** *by Vikram Virdi, UGent/VIB, Ghent, Belgium*

16:50 **Finish Day two**

19:00 **Symposium Dinner in the beautiful Thagaste Monastery in the historic heart of Ghent**

ECMIS
2-5 June 2019, Ghent, Belgium

SCIENTIFIC PROGRAM WEDNESDAY JUNE 5

Chairmen Ann-Mari Svennerholm and Weiping Zhang

New vaccination strategies

9:00 **Keynote Lecture: "New oral vaccination strategies based on the interaction of enterotoxigenic *E. coli* with the intestinal mucosa"** by *Bert Devriendt Ghent University, Belgium*

9:40 **Selected oral presentations**
9:40 "A Phase 1 Dose Escalating Study of a Prototype CS6 Subunit Vaccine with a Modified Heat-labile Enterotoxin from Enterotoxigenic *Escherichia coli* (ETEC)" by Lee T.K., Naval, Medical Research Center, US

10:00 "Intramuscular vaccination with CssBA, a CS6-subunit vaccine candidate against enterotoxigenic *E. coli* (ETEC), and LT(R192G/L211A) as adjuvant promotes antigen-specific α4β7+ antibody-secreting cells" by Milton Maciel, Henry M. Jackson Foundation and Naval Medical Research Center, US

10:20-10:50 **Coffee break**

10:50 **Keynote lecture**: **"New strategies in development of vaccines against ETEC"** *by Weiping Zhang University of Illinois, US*

11:30 **Selected oral presentation**
11:30 "Development of a multivalent, multipathogen conjugate vaccine platform for protection against three major enteric pathogens Enterotoxigenic *Escherichia coli*, Shigella and Campylobacter jejuni" by Laird RM, Henry M. Jackson Foundation for the Advancement of Military Medicine and Naval Medical Research Center, US

Chairman Eric Cox

11:50 **Keynote lecture: "Clinical trials of an oral ETEC vaccine, ETVAX, in children in developing countries and in travelers"** *by Ann-Mari Svennerholm, Department of Microbiology and Immunology, University of Gothenburg, Sweden*

12:30 **Closure and Farewell Lunch**

PART I: INVITED LECTURES

Role of the maternal microbiota in shaping gene expression and microbiota composition in the offspring intestine

Mercedes Gomez de Agüero[1,2], Jakob Zimmermann[1], Cristina Kalbermatter[1], Sandro Christensen[1], Kathy D. McCoy,[1,3], Andrew J. Macpherson[1], Stephanie C. Ganal-Vonarburg[1]

[1]Division of Gastroenterology, Department for BioMedical Research (DBMR), University Clinic for Visceral Surgery and Medicine, University of Bern, 3008 Bern, Switzerland
[2]Institute for Systems Immunology, University of Würzburg, Würzburg, Germany
[3]Cumming School of Medicine, University of Calgary, Calgary, Canada

Postnatal colonization of body surfaces and the intestine with commensal microbes has been assumed to drive postnatal immune development.

Reversible colonization of germ-free mice with an engineered *E.coli* strain allowed transient colonization of germ-free mice exclusively during pregnancy (gestational colonization). The dams then delivered and nursed their pups germ-free and offspring were never exposed to live bacteria. Gestational colonization increased intestinal NKp46$^+$ class 3 innate lymphoid cells (ILC3) and F4/80$^+$CD11c$^+$ mononuclear cells in the pups. Intestinal epithelial transcriptional profiles of the offspring were extensively reprogrammed, including increased expression of genes for antibacterial peptides and metabolism of microbial molecules. These effects were independent of Toll-like receptors, but in some cases were dependent on maternal antibodies that retain microbial molecules and transmit them to the offspring during pregnancy and via milk. Using 13C-isotopically fully-labeled *E. coli* HA107 for gestational colonization and mass spectrometry, we followed specific bacteria-derived metabolites from the mother to the maternal milk and offspring tissues. Gestational bacterial metabolite exchange included natural microbial ligands for the aryl hydrocarbon receptor (AhR). Feeding pregnant mice with the AhR ligand indole-3-carbinol was sufficient to increase intestinal NKp46$^+$ ILC3s in the offspring, revealing the AhR pathway as one important factor in maternal microbiota-dependent shaping of the neonatal immune system. The offspring born to gestationally colonized mothers were primed to limit penetration of intestinal microbes during postnatal colonization and acquired a slightly different endogenous microbiota.

In conclusion, our data demonstrate that the maternal microbiota determine the composition and function of neonatal innate immunity. In a next step of the project, we are addressing if epigenetic modifications induced by metabolites deriving from the maternal microbiota are involved in this prenatal immune priming.

ECMIS
2-5 June 2019, Ghent, Belgium

Cathelicidins, host defense peptides against *E. coli* infection

Maarten Coorens, Viktoria A F Schneider, Tryntsje Cuperus, Maaike R Scheenstra, Albert van Dijk, Edwin J A Veldhuizen, Henk P Haagsman

Division of Molecular Host Defence
Department of Infectious Diseases & Immunology
Veterinary Medicine, Utrecht University
Utrecht, The Netherlands

Cathelicidins are cationic host defense peptides with an important function in the early vertebrate host response against invading pathogens. These peptides are secreted at mucosal surfaces by leukocytes and epithelial cells upon interaction with pathogenic microorganisms. Cathelicidins exhibit direct antimicrobial activity but also have important immunomodulatory activities. A general overview will be given of properties and functions of cathelicidins from different species.

The focus of the presentation will be on avian cathelicidins, particularly CATH-2, one of the four chicken cathelicidins. First, the mechanism of action will be presented of the antimicrobial activity of CATH-2 against *E. coli.* By a combination of live-imaging using confocal fluorescence microscopy and transmission electron microscopy (TEM), including immunogold TEM, a detailed view of the antimicrobial action of CATH-2 could be obtained.

Next, an overview of the immunomodulatory activities of CATH-2 will be given. These include its pro-inflammatory activity by stimulating DNA-uptake by macrophages and subsequent activation of TLR9 (mammals) or TLR21 (birds). This property may be important for vaccine designs. CATH-2 blocks TLR4 activation by LPS and, therefore, also exhibits anti-inflammatory properties. Interestingly, the anti-inflammatory activities of CATH-2 by inhibiting TLR4 and also TLR2 activation occur only if *E. coli* is killed, indicating that cathelicidins are important immunomodulators that dampen the inflammatory response to prevent excessive tissue damage caused by bacterial products.

Finally, the prophylactic and therapeutic properties of (derivatives of) CATH-2 will be presented. It will be shown that *in ovo* administration of CATH-2 protects chickens from colibacillosis after an intratracheal challenge with avian pathogenic *E. coli* at 7 days after hatch. Similarly, prophylactic treatment of zebrafish embryos and mice with CATH-2 (-derivatives) partially protects against bacterial infections. A putative mechanism of action of CATH-2 on the priming of the innate immune system will be presented.

ECMIS
2-5 June 2019, Ghent, Belgium

T-dependent humoral responses in mucosal sites

Meryem Aloulou

CRCN, INSEM, Center for Pathophysiology of Toulouse Purpan, Toulouse University, France

In mammals, the gut is populated by an extremely dense and diverse bacterial community. The colonization of the intestine by bacteria is invariably associated with prompt and abundant generation of IgA by the gut-associated lymphoid tissue (GALT), which involves both T cell-dependent and independent pathways. Diversification of the IgA repertoire is mandatory to maintain gut homeostasis and ensure mucosal defense. The maturation of B cells takes place in germinal centers (GC), where the interaction of B cells with T follicular helper (Tfh) cells elicits antibody affinity maturation and isotypic switch brought about by the expression of activation-induced cytidine deaminase (AID). Tfh cells are thought to be the positive regulators of this process, with T follicular regulatory cells (Tfr), a subset of Foxp3+ regulatory T (Treg) cells, in the role of negative regulators. Gut Treg cells, however, in addition to suppressing inflammation and preserving immune tolerance, are known also to promote GC and IgA responses by generating GC T cells, ultimately resulting in the diversification of gut microbiota. Gut Treg depletion, in fact, causes a rapid loss of specific IgA responses in the intestine. In conclusion, Tfh and Tfr cells function not so much in opposition but in a mutualistic relationship to regulate the GC reaction in the gut, maintain a diverse and healthy gut microbiota, and foster immune homeostasis.

ECMIS
2-5 June 2019, Ghent, Belgium

New insights regarding the interplay between *Shigella* and human lymphocytes

Katja Brunner

Molecular Microbial Pathogenesis
Institut Pasteur, France

The enteropathogen, *Shigella*, is highly virulent and remarkably adjusted to the intestinal environment of its almost exclusively human host. Key for *Shigella* pathogenicity is the injection of virulence effectors into the host cell *via* its type three secretion system (T3SS), initiating disease onset and progression by a vast diversity of secreted T3SS effectors and their respective cellular targets. Well studied do-date are the multifaceted modulations of host signaling pathways exerted by *Shigella* T3SS effectors, which include the subversion of host innate immune defenses and the promotion of intracellular bacterial survival and dissemination. Our group focuses on *Shigella* subversion mechanisms of the adaptive immunity by studying the direct targeting of lymphocytes *via* invasion-dependent and independent mechanisms (Pinaud *et. al.*, PNAS, 2017; reviewed in Brunner *et. al.*, Hum Vaccin Immunother., 2019). For instance, contact with the needle tip protein IpaD induces B-lymphocyte apoptosis in a TLR2-dependent manner (Nothelfer *et. al.*, J. Ex. Med., 2014). In $CD4^+$ lymphocytes, injection of the effector protein IpgD was shown to cause migratory arrest in both, invaded and non-invaded T cells (Konradt *et. al.*, 2011, Cell Host Microbe; Salgado-Pabón *et. al.*, PNAS, 2013). Interestingly, injection-only and invasion were shown to be dependent on specific surface glycans accumulating on the plasma membrane upon T cell activation (Belotserkovsky *et. al.*, mBio, 2018). Finally, recent findings show perturbed immune synapse formation by invaded T cells suggesting further downstream effects on adaptive immunity. Taken together, *Shigella* pathogenicity constituted of invasion-dependent and -independent, T3SS effector-mediated targeting of lymphocytes. Along with consequences on vaccine development, these findings offer new directions for future research endeavors towards a better understanding of immunity to *Shigella* infection.

ECMIS
2-5 June 2019, Ghent, Belgium

Remodeling of intestinal epithelial architecture by enterotoxigenic *E. coli*

Brunda Tumala[1], Alaullah Sheikh[1], Makedonka Mitreva[1], Matthew Ciorba[2], Richard D. Head[3] James M. Fleckenstein[1,]

Divisions of Infectious Diseases[1], and Gastroenterology[2], Department of Medicine, and Department of Genetics[3], Washington University School of Medicine, Saint Louis Missouri. Medicine Service[4], John Cochran VA Medical Center, Saint Louis, Missouri

Enterotoxigenic *Escherichia coli* (ETEC) are a leading cause of diarrheal morbidity. These organisms cause hundreds of millions of illnesses, and tens of thousands of deaths in young children annually. The basic molecular mechanisms underlying development of diarrheal illness associated with ETEC are well-established. Although deaths from infectious diarrhoea appear to have declined over the past several decades, morbidity associated with ETEC has continued unabated.

Notably, enteric infections have been linked to development of environmental enteropathy characterized by functional and morphologic changes in the small intestine resulting in poor nutrient absorption, developmental impairment, and hypo-responsiveness to oral vaccines. ETEC and other diarrheal pathogens have been linked to poorly understood sequelae including malnutrition, growth stunting, and cognitive impairment greatly compounding the impact of these infections.

Interestingly, structural changes including villous blunting identified in the small intestinal mucosa of young children in developing countries with environmental enteropathy are similar to those observed in adults with tropical sprue, a condition of unclear etiology also characterized by impaired nutrient uptake and weight loss.

Recent studies of small intestinal enteroids treated with heat-labile toxin (LT) of ETEC indicate that LT induces substantial transcriptional reprogramming of epithelia. Interestingly, LT up-regulates a receptor on the epithelial surface facilitating bacterial adhesion and toxin delivery. In addition, LT modulates multiple genes that contribute to the structural development of the brush border, resulting in morphologic changes in microvilli characteristic of environmental enteropathy and tropical sprue. Furthermore, we observed down-regulation of multiple solute transporters involved in nutrient absorption, and alterations in the crypt-villous axis maturation program that may also contribute to alteration of the small intestinal epithelia.

While further studies are needed to examine the impact of ETEC and its toxins on intestinal health, these early data provide evidence that these pathogens induce changes that extend beyond those associated with the acute diarrheal illness.

ECMIS
2-5 June 2019, Ghent, Belgium

Bile salts and other host factors regulate expression of ETEC virulence genes

Enrique Joffre[1], Matilda Nicklasson[2], Lei Sun[1], Baoli Zhu[3], Åsa Sjöling[1]

1 Department of Microbiology, Tumor and Cell Biology (MTC), Karolinska Institutet, Stockholm, Sweden
2 Institute of Biomedicine, Dept. Microbiology and Immunology, University of Gothenburg, Gothenburg, Sweden
3CAS Key Laboratory of Pathogenic Microbiology & Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China

Pathogenic bacteria use specific host factors to modulate virulence and stress responses during infection. Enterotoxigenic *Escherichia coli* (ETEC) colonize the epithelium of the small intestine and use colonization factors and secreted toxins to induce disease. We have previously described that bile salts present in the gut specifically up-regulate the colonization factor CS5 in ETEC.

The bile salt response was analyzed using a clinical isolate and its corresponding mutants of colonization factor encoding genes for CS5, CS6, and the putative transcription factor CsvR. RNA seq and Pacific Bioscience (SMRT) sequencing was used to determine the transcriptional response and the plasmid content of the isolate.

We found that CS5-expressing ETEC senses bile salts through a virulence regulon present on a large plasmid. The bile salt glyco-conjugated cholate hydrate (GCH) induced expression of CS5 and additional putative virulence factors while flagellar genes and motility was down-regulated. RNA-seq results on a *csvR* mutant followed by complementation of the gene indicated that the response to bile salts is mediated by CsvR and involves a large number of both chromosomal and plasmid-borne genes. In addition we found that GCH induced aggregation of ETEC.

Several enteric pathogens modulate virulence in response to bile salts. The regulon found in CS5-expressing ETEC is part of a conserved response and homologues to CsvR were found in several enteropathogens and other ETEC lineages. Our results indicate that ETEC use bile salts secreted into the intestine as a signal to turn on colonization factors and down-regulate motility in order to adhere the epithelium.

ECMIS
2-5 June 2019, Ghent, Belgium

Overview of Stx phages diversity and their role in virulence and evolution of *Escherichia coli*

Frederic Auvray

Digestive Health Research Institute (IRSD) : UMR INSERM 1220, INRA 1416, ENVT, UT3

Escherichia coli strains lysogenized with bacteriophages that express Shiga toxin (Stx) can be responsible for human infections, ranging from mild diarrhea to hemolytic-uremic syndrome (HUS). Stx is known as the main virulence factor responsible for HUS development.

Ruminants, particularly cattle, are the primary reservoir for Stx-producing *E. coli* (STEC). Cattle colonization by STEC is considered asymptomatic and human infections arise from exposure to contaminated water or food, and direct animal or person-to-person contact.

Stx bacteriophages show a high degree of variability in their genomic organization, shapes and ability to propagate and express Stx. They carry two different groups of Stx-encoding genes, *sx1* and *stx2*, which are divided into three (a, c, and d) and seven (a to g) subtypes, respectively. Strains that express Stx2 (and especially Stx2a) are more pathogenic than strains encoding only Stx1, or both Stx1 and Stx2. The *stx* genes are located in the phage late gene region, and their expression occurs during the phage lytic cycle which can be induced by DNA damaging agents (including antibiotics) or other factors that trigger the bacterial SOS response.

Induction of Stx phages is not only related to HUS development through the release of toxin, but also to the dissemination of virulence genes through the release of free Stx phages in host, food or the environment. Phage-mediated transfer of *stx* genes plays an important role in the evolution of *E. coli*, as illustrated by the emergence of novel « hybrid » *E. coli* strains carrying *stx* genes and virulence genes from various *E. coli* pathotypes, which pose new threats to human health. Finally, loss of Stx prophages by STEC can occur *in vitro* and *in vivo* in human or animal intestinal tracts, raising diagnostic issues.

ECMIS
2-5 June 2019, Ghent, Belgium

Commensal *E. coli* that enhance toxin production by *Escherichia coli* O157:H7

Edward G. Dudley, Ph.D

The Pennsylvania State University, and Director of *E. coli* Reference Center
University Park, PA 16802. USA.

Escherichia coli O157:H7 is a foodborne pathogen most commonly transmitted to humans when fecal material from cattle comes into contact with food, and the food is subsequently uncooked or undercooked. After colonization of the gastrointestinal tract, the organism can elaborate a toxin called Shiga toxin (Stx), which is necessary for the severe symptoms observed in some patients such as haemolytic uremic syndrome. Previous genetic studies identified several features common to highly virulent *E. coli* O157:H7 strains. For example, such strains often carry the Stx2a allelic variant, and invariably have a type III secretion system called the LEE. Genome analysis has also supported the hypothesis that highly virulence strains fall within defined phylogenetic lineages. More recently, studies from our group and others began to mechanistically describe how members of the gut microbiota may increase Stx2a production. Our data suggests there are at least three mechanisms by which non-pathogenic *E. coli* increase Stx2a production by *E. coli* O157:H7 during co-culture: 1) through infection of the non-pathogenic strain by the *stx2a*-converting bacteriophage, described previously by others; 2) through secretion of colicins and microcins that triggers Stx2a activation; 3) through an undefined mechanism that does not appear to involve the phage or secreted molecules.

ECMIS
2-5 June 2019, Ghent, Belgium

Possible roles of glycoproteins for establishment and persistence of bacteria in the host

Bloch S.[1], Tomek M.B. [1], Belibasakis G.N. [2], Schäffer C .[1]

1 Department of NanoBiotechnology, NanoGlycobiology *unit, Universität für Bodenkultur Wien, Vienna Austria*
2 Division of Oral Diseases, Department of Dental Medicine, Karolinska Institutet, Huddinge, Sweden
christina.schaeffer@boku.ac.at

Introduction: Bacterial glycoproteins are primarily localized in or on the cell envelope, where they serve as a molecular barcode, mediating distinct interactions with the cellular environment and the host and increasing bacterial fitness. Glycosylated fimbriae are one of the primary mechanisms of virulence of, for instance, *E. coli*, *Staphylococcus* sp. and *Streptococcus* sp. The periodontal pathogen *Tannerella forsythia* employs its unique surface (S-) layer glycoproteins as virulence factors. It is important to note that glycans are secondary gene products, which poses challenges to characterizing glycan biosynthesis mechanisms as a basis for lead finding.

Aims: To increase our knowledge on the pathogenesis of periodontal disease we investigated putative roles of the *T. forsythia* S-layer glycoproteins in the establishment of the bacterium within the oral biofilm consortium and the host immune response.

Results: The localization of *T. forsythia* within the biofilm varied depending on changes in the S-layer glycan, which also affected aggregation with and the prevalence of other bacteria present in a multispecies biofilm model. Immune response profiling of primary monocytes and HOK revealed that truncation of the *T. forsythia* glycan leads to significant reduction of IL-1β and regulates macrophage inflammatory protein-1. HOK infected with *T. forsythia* produce IL-1Ra, chemokines and VEGF. Overall, the T. forsythia S-layer and attached sugars contribute to dampening the immune response to initial infection, mediate persistence of the bacterium in the host and, hence, play a pivotal role in orchestrating the bacterium's virulence.

Conclusions:
Our findings support a role of *T. forsythia* cell surface structures in the virulence of this species when interacting with host tissues and the immune system, from within or beyond the biofilm. Overall, a better knowledge of bacterial glycoproteomes and resulting glycan structure-function relationships in combination with metabolic glyco-engineering, can open up new horizons in the discovery and design of new drugs, bioactive compounds, and vaccines.

ECMIS
2-5 June 2019, Ghent, Belgium

Receptor analogues as strategy to prevent *E. coli* infections

Cox Eric[1], Bert Devriendt[1], Arnouts Sven[2], Coddens Annelies, Nguyen Van Ut

[1]Laboratory for Immunology and [2]Provaxs, Faculty of Veterinary Medicine, Ghent University, Belgium

Colibacillosis is a serious problem in piglets, especially in the period shortly after weaning, where it can result in diarrhea and/or edema disease leading to growth retardation and mortality. Control of colibacillosis mainly occurs using antibiotics and ZnO supplementation of feed. Both strategies are under pressure due to increases in antibiotic resistance and impact on the environment. Main causes of post-weaning colibacillosis are $F4^+$ and $F18^+$ enterotoxigenic *Escherichia coli* (ETEC) producing LT, STa and/or STb enterotoxins and shiga toxin Stx2e producing $F18^+$ *E. coli* (STEC). F18 fimbriae are composed of the major subunit FedA and some minor subunits among which FedF is a tipadhesin responsible for interaction with glycosphingolipids of the cell membrane of enterocytes. Our lab demonstrated that FedF interacts with glycosphingolipids having blood group ABH determinants on a type-1 core. The relative binding affinity to different blood group determinants decreases in the order B5 type 1 and A6 type1, A7 type I and B7 type 1, H5 type 1, A7 type 4, A8 type 1 and A9 type 1, with the latter having the weakest interaction (Coddens et al., 2009; *PCT/EP2009/062699; INHIBITORS OF F18$^+$ E COLI BINDING).*

The oligosaccharide A6 type 1 was chosen to determine the percentage inhibition of F18ab *E. coli* to villi. Bacteria were preincubated with the sugar, whereafter they were incubated with the villi of F18R positive pigs. Ten milligram per milliliter was able to decrease binding with ± 73 %. To improve the efficacy of the inhibition, the sugar was conjugated on different carriers and conjugates were tested *in vitro* for inhibition of the F18 *E. coli* binding to villi. One conjugate was able to inhibit binding with 50 to 70% in concentrations of 0.4 to 1.0 μg/ml.

To test if this interaction was able to decrease fluid secretion induced by an infection with an $F18ac^+$ STa^+STb^+ *E. coli* strain the small intestinal segment perfusion (SISP- model was used. The conjugate in a concentration of 10 μg/ml could prevent fluid secretion by the ETEC strain.

In an *in vivo* experimental infection, this dose was used in feed or in drinking water and significantly reduced the duration and height of the fecal excretion of the $F18ab^+STx2e^+$ *E. coli* strain F107/86. Field trials have been performed on 2 farms: one with and $F18ab^+STa^+$ STx2e+ *E. coli* infection and another with a $F18ac^+STa^+LT^+$ *E. coli* infection. Results of both studies are not yet available.

ECMIS
2-5 June 2019, Ghent, Belgium

In feed administered non-encapsulated monomeric porcine IgA antibodies produced in yeast as prophylaxis against F4-mediated colibacillosis in pigs

Vikram Virdi[1,2], Jorge Palaci[3,4], Bram Laukens[1,2], Eric Cox[5], Ann Depicker[3,4], & Nico Callewaert[1,2]

[1]Department of Biochemistry and Microbiology, Ghent University, 9000 Gent, Belgium.
[2]VIB Center for Medical Biotechnology, 9052 Gent, Belgium.
[3] Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Gent, Belgium.
[4] VIB Center for Plant Systems Biology, 9052 Gent, Belgium.
[5]Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke, Belgium.

Oral passive immunization with antibodies against specific gastro-intestinal (GI) pathogens can provide immediate protection at the mucosal frontier. However, choosing an efficient and stable antibody isoform for oral delivery at the GI tract remains elusive. A rational choice is the predominant immunoglobulin (Ig) isoform at the mucosal surface, i.e. the IgA, existing exclusively as secretory IgA (SIgA). The commercial manufacturing of SIgAs is difficult in conventional expression systems, as SIgAs are complex hetero-decameric antibodies. Based on the camelid single-domain antibodies, we previously designed simpler hetero-hexameric SIgAs and the precursor formats – dimeric and monomeric IgAs– and expressed these to high amounts in *Arabidopsis* seeds. Unpredictably, we now demonstrate that the monomeric IgA (mVHH-IgA) format against enterotoxigenic *Escherichia coli* (ETEC) delivered orally in feed is sufficient to prevent the ETEC bacterial attachment and to lower the shedding of the challenge ETEC bacteria, thus protecting piglets similarly as the SIgA format. Furthermore, we show that mVHH-IgAs can be produced efficiently in soybean seeds and the well-known *Pichia pastoris* yeast cell production platform. Crushed soybean seeds expressing mVHH-IgA, or the dried medium from *Pichia* secreting mVHH-IgA, when orally delivered in a feed formulation, protected the piglets from the ETEC challenge. The convenient scalability and frugal downstream processing make these anti-ETEC mVHH-IgAs most suitable for translation as a safe alternative prophylaxis to antibiotics, for preventing ETEC-related recurrent economic losses in the porcine industry. Moreover, given the anatomical organ size similarity, these in-piglet model results are highly relevant for translation of oral mVHH-IgA applications for human GI infections.

ECMIS
2-5 June 2019, Ghent, Belgium

New oral vaccination strategies based on the interaction of enterotoxigenic *E. coli* with the intestinal mucosa

Bert Devriendt

Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke, Belgium

Most pathogens invade the host at the mucosal surfaces, such as the gut. To protect against gut-dwelling pathogens a local intestinal immune response at the site of entry is required. This protective immunity usually comprises pathogen-specific secretory IgAs that can prevent the initial attachment of pathogens to the mucosal surfaces or neutralize their enterotoxins. Despite this knowledge, most of our current vaccines are administered systemically, which does not result in a robust intestinal immunity as this strategy fails to induce gut-homing receptors on activated lymphocytes. In contrast, to mount protective intestinal immunity, vaccines should be delivered to the intestinal immune system via oral administration. Most evaluated oral vaccines are based on inactivated or live-attenuated pathogens. However, inactivated vaccines often lack efficacy, while live-attenuated vaccines have other drawbacks, like a risk of reversion to virulence and their environmental dissemination. These shortcomings have shifted the focus of oral vaccine design to subunit vaccines.

The development of oral subunit vaccines is however challenging due to several well-developed barriers in the gut. On top of the harsh environment of the stomach and small intestine (low pH, digestive enzymes, and bile salts) that easily destroys vaccines and the tolerogenic mechanisms pervading the intestinal tissues, the intestinal epithelial barrier restricts uptake of macromolecules. These barriers result in a low efficacy of oral subunit vaccines. Hence, novel innovative strategies are required to enhance the delivery of vaccine antigens to intestinal immune system. Here, we show that this development is accelerated by using insights gained from investigating the interaction of enterotoxigenic *E. coli* with the small intestinal epithelium. In the past, we reported that F4 fimbriae of porcine ETEC strains display a potent oral immunogenicity, which relies in part on their interaction with aminopeptidase N (APN). This metallopeptidase is present on the apical membrane of small intestinal epithelial cells. Interestingly, selective delivery of antigens or microparticles by APN-specific antibodies resulted in an enhanced transport through the intestinal epithelial and elicited immune responses in piglets. We further built on these findings and generated different APN-specific antibody formats, including monoclonal antibodies, porcinized monoclonal IgA antibodies and fusion constructs comprising llama-derived VHHs and IgG Fc domains. Upon their production, purification and *in vitro* validation, these different antibody formats were shown to be taken up by small intestinal epithelial cells in gut ligated loops and to elicit systemic and mucosal immune responses upon oral administration in a piglet model.

Further validation of these antibody formats in a challenge infection model is however required to evaluate the efficacy of APN-mediated selective targeting of oral subunit vaccines to elicit protective intestinal immunity in pigs.

ECMIS
2-5 June 2019, Ghent, Belgium

New strategies in development of vaccines against ETEC

Weiping Zhang

Department of Pathobiology, University of Illinois at Urbana-Champaign, Illinois, USA

Enterotoxigenic *Escherichia coli* (ETEC) remains a leading cause of in young animals, children and travellers. Vaccination is considered an effective and practical prevention approach against ETEC. However, developing ETEC vaccines has encountered technical challenges, mainly because of virulence factor heterogeneity and the difficulty in inducing protective immunity against a key toxin virulence factor.

Multiepitope-fusion-antigen (MEFA), an epitope- and structure-based vaccine technology, suggests the feasibility in developing broadly protective vaccines for ETEC. By using a backbone immunogen to present neutralizing epitopes from heterogeneous virulence factors and to mimic epitope native antigenicity, MEFA enables a single protein to induce broadly protective immunity, facilitating the development of broadly effective multivalent vaccines.

By applying MEFA vaccinology, we constructed an adhesin-toxin MEFA which uses LT backbone and carries neutralizing epitopes from ETEC fimbriae K88 and F18 and toxins including STa, STb and Stx2e, demonstrated adhesin-toxin MEFA-induced antibodies broadly protective against K88 & F18 fimbriae and four toxins (LT, STa, STb, Stx2e), and accelerated the development of a broadly protective vaccine for pig post-weaning diarrhea (PWD). MEFA technology also assisted the construction of human ETEC multivalent antigens. CFA/I/II/IV MEFA which has human ETEC adhesin CFA/I subunit CfaB as the backbone to present epitopes of adhesins CFA/II (CS1, CS2, CS3) and CFA/IV (CS4, CS5, CS6) induced antibodies inhibiting adherence of the seven most important ETEC adhesins associated with diarrhea. Toxoid fusion 3xSTa_{N12S}-$mnLT_{R192G/L211A}$ which uses LT toxoid monomer $mnLT_{R192G/L211A}$ backbone to host three STa toxoid STa_{N12S} induced antibodies neutralizing both enterotoxins (LT, STa) and protecting against ETEC diarrhea in a pig challenge model.

Results revealed MEFA an effective platform for the development of broadly protective vaccines against ETEC diarrhea, suggesting the potential application of MEFA vaccinology for developing vaccines against other pathogens or diseases.

ECMIS
2-5 June 2019, Ghent, Belgium

Clinical trials of an oral inactivated ETEC vaccine (ETVAX) in children in developing countries and in travelers

Ann-Mari Svennerholm, Anna Lundgren, Firdausi Qadri et al

Department of Microbiology and Immunology Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden, icddrb , Dhaka, Bangladesh

We have previously developed an oral inactivated multivalent *Escherichia coli* (ETEC) vaccine (ETVAX) for use in children in developing countries and in travelers. Following intensive studies of the vaccine for safety and immunogenicity, including capacity to induce immunological memory, in adult Swedish volunteers the vaccine has been tested in a large Phase I/II trial in descending age groups in Bangladesh (495 subjects). Four different groups received two vaccine doses (in different dosages) alone or with different amounts of dmLT adjuvant or placebo (buffer only) two weeks apart: adults 18-45 years and children 24-59 months, 12-23 months and 6-11 months. Blood and feces were collected from all subjects before the first dose and then 7, 19 days and 28 days (feces only) after vaccination. Mucosal immune responses against all key vaccine antigens (i.e. CFA/I, CS3, CS5, CS6 and LTB) were studied in all age groups using the IgA antibody in lymphocyte secretions (ALS) assay and in younger infants also by measuring secretory IgA (SIgA) antibody responses in fecal extracts Since only small volumes of ALS samples could be collected, especially from the younger cohorts, an electro-chemiluminescence assay (MSD) was used.

The vaccine was safe: the only significant side-effect observed was mild vomiting in a low percentage of children in the youngest age groups when given fractionated doses (1/4 or 1/8 of a full dose). Determination of ALS responses revealed that 100% of the vaccinated adults responded to all five key vaccine antigens, whereas only a few placebo recipients responded to a single antigen (responses in the Bangladeshi adults were higher and more frequent than in the Swedish adults). Encouragingly, ALS responses in Bangladeshi children (2-5 years old) were similar in frequency and magnitude to those seen in Bangladeshi adults. Also a majority of children in the 12-23 months group mounted good ALS responses to the vaccine. However, in infants 6-11 months old ALS responses against the CFs (15-40%) and in particular O78LPS (<5%) were comparatively infrequent and of lower magnitudes than in older children and adults, whereas anti-LTB responses were more comparable across the different age groups. However, ca 50% of the infants responded with fecal SIgA responses against all the CFs and O78 LPS suggesting that ALS analyses are not optimal for determining mucosal immune responses against bacterial antigens in infants. At variance with responses in the older pediatric and adult groups, a higher percentage of placebo recipients in the infant cohort also had increased immune responses to key antigens, which may suggest a high incidence of asymptomatic infections in infants. No significant differences in response rates were noted among children given different doses of vaccine (1/8; ¼; ½ versus full adult dose) or vaccine +/- dmLT adjuvant.

A study has also been undertaken by Anu Kantele, Helsinki et al, to evaluate ETVAX for safety, immunogenicity and protective efficacy against ETEC disease in 750 Finnish

travelers to Benin. Healthy adults have been given two doses of either ETVAX or placebo in a double-blinded manner at least one week before travel and occurrence of moderate – severe episodes of diarrhea with ETEC as sole pathogen determined in feces during a two week stay in Benin and during the first week after return to Finland. The results of the study will be available during early autumn 2019.

A Phase I trial has also been initiated in Zambia by Roma Chilengi et al as to evaluate the optimal dosages of ETVAX identified in the Bangladeshi trial (1/4 and an 1/8 of a full dose + a low dose of dmLT adjuvant) in children 6-23 months. This study will be followed by a Phase 2B children in 6-23 months old children in the Gambia.

PART II: SELECTED ORAL PRESENTATIONS

Human cellular and humoral immune responses against colonization factors and mucinase YghJ after experimental infection with an epidemiologically relevant STh-only ETEC strain.

Sakkestad ST.[1], Steinsland H. [2,3], Sommerfelt H. [4,5], Hanevik K. [1,6]

1 Department of Clinical Science, Faculty of Medicine, University of Bergen, Bergen, Norway
2 Centre for International Health, Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway
3 Department of Biomedicine, University of Bergen, Bergen, Norway
4 Centre for Intervention Science in Maternal and Child Health (CISMAC), Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway
5 Norwegian Institute of Public Health, Oslo, Norway
6 Norwegian National Advisory Unit on Tropical Infectious Diseases, Department of Medicine, Haukeland University Hospital, Bergen, Norway
kurt.hanevik@med.uib.no

Introduction: Infections with enterotoxigenic *Escherichia coli* (ETEC) expressing the heat-stable toxin (STh) are important causes of moderate-to-severe diarrhea in children in low- and middle-income countries. ETEC colonization factors and *E. coli* mucinase YghJ are important targets for developing ETEC vaccines.

Aims: We aimed to evaluate human cellular and humoral immune responses, kinetics and duration against the epidemiologically relevant ETEC strain TW10722 (CS5, CS6, STh). Healthy adult volunteers (n=21) ingested TW10722 doses ranging from 10^6 to 10^{10} colony-forming units (CFU). Blood samples were obtained before infection, and on day 10, day 28, 6 months, 1 year and 2 years after infection. Levels of antigen-specific IgG and IgA antibodies were measured in a multiplex bead-based flow cytometric immunoassay. Activated CD4 T cells were measured with a 2-day whole blood assay with stimulation by recombinant CS5, CS6, or YghJ structural proteins. CD4+ T cells co-expressing CD25 and CD134 were counted by flow cytometry.

Results: At the highest inoculum 10^{10} CFU we observed an overall diarrhea attack risk of 78%. We found a 5.5-fold increase in CS5-specific activated CD4+ T cells, from 0.5% before infection to 2.6% on day 10 ($p<0.0001$), which remained significantly elevated after 28 days and 6 months, and, in several volunteers, even after 1 and 2 years. Similar trends, albeit weaker, were seen for CS6 and YghJ. Serum antibody responses against YghJ peaked on day 10, while colonization factor responses peaked on day 28. Results from multiple linear regression analyses showed that increased antigen-specific antibody and CD4+ T cell responses were associated with diarrheal disease among the volunteers, but not with inoculum dose.

Conclusions: Experimental ETEC infection with TW10722 elicited rapid and long lasting human CD4 T cell responses against CS5, CS6, and mucinase YghJ. The kinetics between serological responses to colonization factors CS5 and CS6 differed from that of secreted mucinase YghJ.

ECMIS
2-5 June 2019, Ghent, Belgium

Porcine small intestinal enteroids as a model to explore host-pathogen interactions

Vermeire B.; Janssens R. J. J.; Cox E.; Devriendt B.

Laboratory of Immunology, Department of Virology, Parasitology, Immunology, Faculty of Veterinary Medicine, Ghent University
Bjarne.Vermeire@Ugent.be

Introduction: Enterotoxigenic *E. coli* infections still represent a considerable disease burden in man and livestock. Despite intensive research efforts, many aspects of the interaction of this pathogen with the gut epithelium are still lacking due to inadequate models. Enteroids represent a valuable model to study host-pathogen interactions at the epithelial surface. Much research has been done on murine and human enteroids, but only a handful studies evaluated the development of enteroids in other species. Porcine enteroid cultures have been described, however little is known about the functional responses to specific pathogens or their associated virulence factors.

Aims: To develop and characterize a 3D small intestinal epithelial stem cell culture model to study the interaction of enterotoxigenic *E. coli* with the small intestinal epithelium.

Results: We show the development and culture of duodenal, jejunal and ileal crypts into complex enteroids. The presence of progenitor and epithelial stem cells was confirmed in the enteroids by a Sox9 staining, which showed a distinct localization in the crypt-like buds of the more complex structures. These porcine small intestinal enteroids respond in a similar manner as *in vivo* gut tissues to enterotoxins derived from enterotoxigenic *E. coli*. Upon enterotoxin stimulation, enteroids not only displayed a similar dysregulated electrolyte and water balance as shown by their acute swelling, but also secreted inflammation markers such as interleukin 8, as shown in previous studies using porcine intestinal epithelial cell lines.

Conclusions: Enteroids are a promising model to study host-pathogen interactions in the gut, as these better represents the 3D microarchitecture of the small intestinal epithelium and closely mimic *in vivo* intestinal epithelial responses to enteropathogens. New insights obtained in this model might accelerate the design of veterinary drugs for oral use able to control enteropathogens.

ECMIS
2-5 June 2019, Ghent, Belgium

Development of a multivalent, multipathogen conjugate vaccine platform for protection against three major enteric pathogens Enterotoxigenic *Escherichia coli, Shigella and Campylobacter jejuni*

Renee M. Laird[1,2], Milton Maciel Jr.[1,2], Steven T. Poole[1,2], Michael G. Prouty[2], Stephen J. Savarino[2], Mario A. Monteiro[3], Patricia Guerry[2] and Frédéric Poly[2]

[1]Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, [2]Enteric Diseases Department, Naval Medical Research Center, Silver Spring, MD, [3]Department of Chemistry, University of Guelph, Guelph, Ontario, Canada

Introduction: Enterotoxigenic *Escherichia coli* (ETEC), *Shigella* spp. and *Campylobacter jejuni* are major causes of bacterial diarrhea worldwide. Various subunit and live attenuated vaccine platforms against individual pathogens are being developed and tested in preclinical and clinical stages.

Aims: We have developed a conjugate vaccine platform which combines surface-exposed polysaccharide antigens from *C. jejuni* and *Shigella* with adhesin proteins from ETEC. We have synthesized three conjugate vaccines using two ETEC recombinant adhesin proteins (CfaEB from CFA/I and CssBA from CS6) which function as carrier proteins for two *C. jejuni* capsules (CPS; HS3 and HS23/36 types) and lipopolysaccharide (LPS) from *Shigella flexneri* 2a.

Results: All vaccines were immunogenic in mice as monovalent, bivalent and trivalent formulations. Antibodies were generated against both polysaccharide and protein components and functional antibodies capable of inducing hemagglutination inhibition (HAI) of H10407 were present in all mice receiving vaccines containing CfaEB. Efficacy of a monovalent vaccine containing *C. jejuni* HS23/36 CPS conjugated to CfaEB (HS23/36-CfaEB) was tested in *Aotus nancymaae* non-human primates (NHPs). Two weeks after the third immunization, all animals were challenged with H10407. Nine weeks after the third immunization and seven weeks after the ETEC challenge, all animals were challenged with HS23/36^{+} *C. jejuni*. All NHPs vaccinated with HS23/36-CfaEB developed anti-CPS and anti-CFA/I antibodies. Importantly, HAI activity against ETEC and serum bactericidal activity against *C. jejuni* were induced. HS23/36-CfaEB vaccinated NHPs were protected against both ETEC and *C. jejuni* diarrhea compared to the PBS immunized control animals.

Conclusions: These data provide promising evidence that a conjugate vaccine platform is feasible for a multipathogen, multi-serotype vaccine against these three enteric pathogens. Future studies will test conjugate dose optimization, expanded multivalent formulations and alternate adjuvant combinations. HS23/36 CPS and CfaEB components have been separately cGMP manufactured and would be available for conjugation and testing in a Phase I study awaiting financial support.

ECMIS
2-5 June 2019, Ghent, Belgium

Vitamin K influences the virulence potential of Enterohemorrhagic Escherichia coli

Kijewski A. [1], *Lindbäck .T*[1], *Rønning H.T* [2], *Aanrud S.G1, Wasteson Y.*[1], *Witsø I.L.*[1] , *L'Abée-Lund T.M.*[1] *and Aspholm M.*[1]

[1] Department of Food Safety and Infection Biology, Faculty of Veterinary Medicine, Norwegian University of Life Sciences
[2] Matriks AS
anne.kijewski@nmbu.no

Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) is the cause of foodborne outbreaks, hospitalizations and deaths worldwide. Today, there is no optimal treatment for EHEC infections, as antimicrobials may exacerbate production of Shiga toxin (Stx), the most important virulence factor of EHEC. Vitamin K is a biomolecule present in the intestinal tract of humans and ruminants. Cattle and small ruminants are healthy reservoirs for EHEC. While vitamin K levels generally are high in ruminants, they vary in the human intestine due to factors such as age, diet and intestinal microbiota. These factors have also been suggested to impact the outcome of EHEC infections.

Aims: To address the influence of the different vitamin K types; phylloquinone, menaquinone, menadione and menadione sodium bisulfite, has on growth and virulence potential of EHEC.

Results: The presence of phylloquinone and menaquinone had minor effects on the growth of EHEC O157:H7 EDL933, while menadione and menadione bisulfite inhibited growth in Luria Bertani broth. Levels of Stx and transcription of the *stx* gene were significantly lower in EHEC grown in the presence of sub-inhibitory levels of vitamin K when induced by ciprofloxacin and H2O2. There was also a concomitant 2 to 25 times reduction in phage production in vitamin K treated cultures. Ciprofloxacin and mitomycin C-induced cellular filamentation was inhibited during growth in cultures containing menadione and menadione sodium bisulfite. Menadione and menadione sodium bisulfite also increased survival of EHEC in the presence of H2O2 and ciprofloxacin.

Conclusions: Our results indicate that vitamin K has a reducing effect on important virulence traits such as toxin and phage production, filamentation and survival in the presence of phage–inducing compounds. Together the results suggest that vitamin K may play a role in EHEC virulence and carriage.

ECMIS
2-5 June 2019, Ghent, Belgium

A Phase 1 Dose Escalating Study of a Prototype CS6 Subunit Vaccine with a Modified Heat-labile Enterotoxin from Enterotoxigenic Escherichia coli (ETEC)

Lee TK[1], Porter CK[1], Gutierrez RL[1], Jaep K[1,2], Poole ST[1,2], Lane AB[3], Hamer M[4], Vazquez, AD[4], Riddle MS[5], Maciel Jr. M[1,2], Dori, KE[1,2], Yang, L[1,2], Alcala, AN[1,2], Tribble, DR[5], Erdem, R[6], Maier, N[6], Bourgeois AL[6], Prouty MG[1]

[1]Naval Medical Research Center, Silver Spring, MD USA; [2]Henry M. Jackson Foundation, Bethesda, Maryland, USA; [3]Walter Reed National Military Medical Center, Bethesda, MD USA; [4]Walter Reed Army Institute of Research, Silver Spring, MD; [5]Uniformed Services University of the Health Sciences, Bethesda, MD; [6]PATH, Washington, D.C.

Introduction: Enterotoxigenic *E. coli* (ETEC) is a leading bacterial cause of diarrhea in developing countries and travelers. ETEC adhere to the intestinal epithelium via colonization factors (CF). As part of a strategy, we have developed subunit vaccines based on the most prevalent colonization factors (CFs) to include CssBA, a recombinant derivative of the globally prevalent ETEC CF CS6. We have performed a phase 1, dose-escalating clinical trial of CssBA with the double mutant heat-labile enterotoxin (LT(R192G/L211A), dmLT) from ETEC (NCT03404674).

Aims: Evaluate the safety and immunogenicity of CssBA ± dmLT given in 3 intramuscular (IM) injections 3 weeks apart.

Results: A total of 50 subjects were enrolled into the study (five cohorts, ten subjects each). CssBA and dmLT were initially administered individually at low dose of 5μg and 0.1μg respectively (n=5 for each product). CssBA and dmLT were subsequently combined and dose-escalated, dmLT to 0.5μg and CssBA 15μg and then 45μg. Prior to dose escalation between cohorts, safety assessments 7 days post the third vaccination were completed. Subjects were actively monitored for safety for 28 days following the last vaccination. Immune responses were assessed with blood, stool, and saliva collections. The only vaccine-related AEs of note were local site reactions including pain (26.0%), tenderness (42.0%), redness (70.0%), and induration (34.0%). The majority (97.0%) of these were of mild severity. There were significant increases in local site reactogenicity with repeated dosing of the vaccine as well as with increased dose of the vaccine. There were no vaccine serious adverse events or unanticipated events.

Conclusions: While vaccine site reactions were common, and increased in frequency at higher doses and later in the vaccine schedule, both CssBA and dmLT were safe and well-tolerated at all dose levels given IM. This is the first use of dmLT as an adjuvant for an ETEC antigen by the IM route.

ECMIS
2-5 June 2019, Ghent, Belgium

Escherichia coli ST131: a versatile multidrug-resistant pathogen in and outside the gut

Yaoqin Hong[1], Sohinee Sarkar[1], Anu Chacko[1], Carola Venturini[2], David Gordon[3], Anthony Verderosa[1], Makrina Totsika[1]

[1] Institute of Health and Biomedical Innovation, School of Biomedical Sciences, Queensland University of Technology, Brisbane, Australia
[2]Centre for Infectious Diseases and Microbiology, Westmead Institute for Medical Research, Sydney, Australia
[3]Research School of Biology, ANU College of Science, Australian National University, Canberra, Australia

Introduction: The WHO has recently declared antimicrobial resistance a new political priority, with *Enterobacteriacea* displaying carbapenem and 3rd generation cephalosporin resistance listed as 'Critical Priority' pathogens for new drug research and development. *Escherichia coli* sequence type 131 (ST131) is the predominant cause of multidrug resistant urinary tract infections (UTIs) and sepsis worldwide. Our previous work has focussed on extra-intestinal pathogenic mechanisms of *E. coli* ST131, identifying key drivers of disease and demonstrating their potential as novel therapeutic targets.

Aims: Here we investigated the intestinal lifestyle of *E. coli* ST131 by examining the capacity of a geographically and genetically diverse set of clinical ST131 isolates to interact with intestinal epithelia and persist in the mammalian gut *in vivo*.

Results: ST131 strains adhered to and invaded into human intestinal epithelial cells more than probiotic and commensal *E. coli* strains. The reference ST131 strain EC958 established persistent intestinal colonisation in mice and expression of type 1 fimbriae enhanced colonisation levels without signs of pathology. A distinct set of clinical ST131 isolates were found to have evolved intestinal pathogenicity via the acquisition of genetic elements from the enteroaggregative *E. coli* (EAEC) pathotype. Using cell and mouse models of infection we showed that these 'hybrid' strains are proficient pathogens both inside and outside the gut.

Conclusions: ST131 strains can efficiently colonise the mammalian gut and persist long-term. Type 1 fimbriae enhance ST131 intestinal colonisation suggesting that mannosides, currently developed as therapeutics for bladder infections and Crohn's disease, could also be used to limit intestinal ST131 reservoirs. This is important when considering the recent emergence of hybrid ExPEC-InPEC pathotypes within this already problematic multidrug resistant *E. coli* lineage.

ECMIS
2-5 June 2019, Ghent, Belgium

The pathogenic mechanism of QS-1 in Avian Pathogenic *E.coli*

*Yang Yang[1,2], Qiangde Duan[1,2], Pengpeng Xia[1,2], Guoqiang Zhu[1,2]**

1. College of Veterinary Medicine, Yangzhou University, Yangzhou 225009, China
2. Jiangsu Co-Innovation Center for Important Animal Infectious Diseases and Zoonoses, Joint International Research Laboratory of Agriculture and Agri-Product Safety of Ministry of Education of China, Yangzhou 225009, China
yzgqzhu@yzu.edu.cn

Introduction: Avian colibacillosis is one of the most serious bacterial diseases caused partly or entirely in poultry industry and presents acute or chronic avian pathogenic *Escherichia coli* (*E. coli*) infectious bacterial diseases in poultry. The virulence factors of APEC lead to the pathogenesis, and Quorum Sensing (QS) system is actively involved in the regulation process of virulence factors.
The signaling molecules in QS are known as autoinducer. In QS-I, *E. coli* encodes a single LuxR homolog named SdiA, but does not express the LuxI homolog, the acyl-homoserine lactone (AHL) synthase, which produces AI-1. APEC can regulate its virulence gene expression in response to exogenous AHLs, but QS-I regulatory mechanism remain to be known.
This study targets APEC CE129 isolate as the reference strain, and *Yersinia enterocolitica yenI* gene was cloned, transferred and expressed into APEC CE129. The pSB01 biosensors as reporter strain was used to screen and confirm the C6-AHL production of CE129. CE129 SdiA mutant strain with in-frame sdiA gene deletion was constructed by λRed recombination system, which lost the ability to sense AHL. On this basis, QS-1 function in APEC was well clarified.

Aims: To explore the QS-1 function upon virulence and discuss regulation mechanism of QS-1 autoinducer AHL signals in APEC strain.

Results: The results showed that QS-1 influence QS-II activity in APEC, as well as adherence and invasion ability. Biofilm formation of APEC was also suppressed under AHL signals. *lsrR*, the important gene in QS-II, decreased by 46.6%. Interestingly, APEC strain showed different phenotypes of acid tolerance and flagella expression, compared with *Enterotoxigenic Escherichia coli*. This find brings us deeper thoughts in QS mechanism.

Conclusions: QS-Iinfluenced biofilm formation, invasion and the adhesion ability of APEC strain, and activate QS-IIsystem. This study explored the regulation mechanism of QS in the virulence of APEC, and laid the foundation for further clarifying the complex mechanism of QS.

ECMIS
2-5 June 2019, Ghent, Belgium

Quorum sensing signal Acyl-Homoserine Lactones enhance the acid resistance of Enteropathogenic *Escherichia coli* O157:H7 by activating the *rpoS* and *gad* system

Zhendong Li[1,2], Boyu Jiang [1,2], Yang Yang[1,2], Guoqiang Zhu[1,2]

1. College of Veterinary Medicine, Yangzhou University, Yangzhou 225009, China
2. Jiangsu Co-Innovation Center for Important Animal Infectious Diseases and Zoonoses, Joint International Research Laboratory of Agriculture and Agri-Product Safety of Ministry of Education of China, Yangzhou 225009, China
yzgqzhu@yzu.edu.cn

Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 can survive passage through the extremely acidic conditions (pH 2~3) from the stomach out of the blue due to its robust acid resistance (AR) systems. The AR of *E. coli* is majorly controlled by the *gad* genes which can be upregulated by type I quorum sensing (QS) autoinducers –Acyl-Homoserine Lactones (AHLs). Although *E.coli* cannot synthesizes AHLs, the AR is activated by the AHLs which are produced by other kinds of bacteria in living environment. We try to target the regulatory mechanism of *E. coli* AR under AHLs.

Aims: To evaluate increased survival rates of EHEC affected by different AHLs species and to investigate regulatory pathway of AHLs on AR systems.

Results: In this study, we found that the exogenous AHLs can obviously enhance the survival rates of EHEC O157:H7 EDL933 at the concentrations of 100uM and 10uM. It is worthwhile that 3oxo-C6-HSL have a more significantly function in AR than C4-HSL and C6-HSL. We engineered EHEC to synthesize AHLs via expressing the AHL synthase gene yenI of *Yersinia enterocolitica*, which confers EHEC the ability to endogenous producing AHLs. According to the transcriptional analysis study, the transcription of *sdiA*, *gadA*, and *rpoS* increased by both exogenous and endogenous (yenI) AHLs. This result showed an intrigued pathway that AHLs enhance the AR of *E. coli* by activating the expression of rpoS, and this modulation could be mediated by the two-component system *uvrY/barA*, because we found that AHLs can activate the *uvrY/barA* which is a well-known positive regulator of *rpoS*.

Conclusions: These findings showed QS-I signal AHLs can enhance the survival level of EHEC in extremely acidic environment via activating the global stress regulator RpoS and *gad* genes. Thus, EHEC infection come with other bacteria in food or stomach helps EHEC eavesdrop on AHLs to greatly enhance its acid response.

ECMIS
2-5 June 2019, Ghent, Belgium

Antibodies derived from STatoxoid-mnLTR192G/L211A toxoid fusions induce neutralizing antibody against LT and STa but show little cross-reactivity with guanylin or uroguanylin

Duan Q.D.[1], *Huang J.C.*[2], *Xiao N.*[2], *Seo H.*[2], *Zhu G.Q.*[1], *Zhang W.P.*[3*]

1. College of Veterinary Medicine (Institute of comparative medicine), Yangzhou University, Yangzhou, China
2. Department of Diagnostic Medicine/Pathobiology, Kansas State University College of Veterinary Medicine, Manhattan, Kansas, USA
3. Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA
dqd@yzu.edu.cn

Introduction: Considering heat-labile enterotoxin (LT) and Heat-stable toxin (STa) are important virulence determinants of enterotoxigenic *Escherichia coli* (ETEC) strains that caused children's and travelers' diarrhea, therefore antigens that induced neutralizing anti-LT and anti-STa antibodies could prevent ETEC infection. Guanylin and uroguanylin are structurally and functionally similar to STa. Like STa, these two important guanylate cyclase C (GC-C) ligands regulate homeostasis in human intestinal and renal epithelial cells. Recent using a 3×STaN12S -mnLTR192G/L211A (monomeric LTR192G/L211A mutant) toxoid antigenwas demonstrated to induce neutralizing anti-LT and STa antibodies, however, concern remains regarding whether the anti-STa antibodies derived from STa toxoids cross-react with guanylin and uroguanylin.

Aims: To develop safe STatoxoid-mnLTR192G/L211A toxoid fusions that induced neutralizing antibody to both LT and STa, but show little or no cross-reactivity with guanylin and uroguanylin.

Results: Western blot analysis showed that the 3×STaN12S-mnLTR192G/L211A, 3×STaL9A/N12S- mnLTR192G/L211A, 3×STaN12S/A14T-mnLTR192G/L211A and 3×STaL9A/N12S/A14H-mnLTR192G/L211A toxoid fusions can be detected by both anti-CT and anti-STa antibodies; and *in vitro* enterotoxicity assay indicated that all of the fusions are lack of LT and STa enterotoxicity. Mice subcutaneously immunized with each above fusion protein developed highly anti-LT and anti-STa antibody responses, and the antibodies derived from 3×STaN12S-mnLTR192G/L211A, 3×STaL9A/N12S- mnLTR192G/L211A and 3×STaN12S/A14T-mnLTR192G/L211A toxoid fusions neutralized LT stimulation of intracellular cAMP and STa stimulation of intracellular cGMP in T-84 cells. Competitive STa enzyme-linked immunosorbent assays (ELISAs) showed that serum mice immunized with 3×STaL9A/N12S- mnLTR192G/L211A, 3×STaN12S/A14T-mnLTR192G/L211A, or 3×STaL9A/N12S/A14H-mnLTR192G/L211A showed little cross-reactivity with guanylin or uroguanylin, whereas serum samples of mice immunized with 3×STaL9A/A14H-mnLTR192G/L211A showed a low level of reactivity with guanylin peptides.

Conclusions: These results demonstrated that antibodies derived from 3×STaN12S-mnLTR192G/L211A, 3×STaL9A/N12S-mnLTR192G/L211A, or 3×STaN12S/A14T-mnLTR192G/L211A neutralized LT and STa *in vitro*, but show little or no cross-reacting with guanylin and uroguanylin. Thus, these toxoid fusions could be used in future ETEC vaccines development.

ECMIS
2-5 June 2019, Ghent, Belgium

Inactivation of *stx*-phages by probiotic *E. coli* strain Nissle 1917

Soundararajan M.[1], Bury S.[1], Förstner K.U.[2], Bünau R.[3], Oelschlaeger T.A.[1]

[1]*Institute for Molecular Infection Biology, Wuerzburg, Germany*
[2] *ZB MED - Information Centre for Life Sciences, Cologne, Germany*
[3]*Pharma Zentrale GmbH, Herdecke, Germany*

Introduction: The probiotic *E. coli* strain Nissle 1917 (EcN) is the active component of gastrointestinal medication, Mutaflor. We have shown that EcN reduces the Shiga toxin (stx) level and growth of shiga toxin producing *E. coli* (STEC) strains in co-culture. STEC strains harbor prophage(s) which encode and express *stx*. These phages can be activated and subsequently infect commensal bacteria thereby converting them into stx producers, life-threatening pathogens.

Aims: To show EcN's resistance to and interaction with *stx*-phages as a prerequisite for using EcN as a safe alternative or supplementary medication for the treatment of STEC infections.

Results: EcN demonstrated a complete resistance towards lysis and lysogeny by *stx*-phages, which was proven by PCR and phage plaque assays. The resistance was traced back to the expression of a phage repressor (*pr*) gene of a defective lambdoid prophage in EcN. Furthermore, incubation of *stx*-phages with EcN resulted in complete inactivation of these phages after 44 h. Various approaches to determine the factor(s) which are involved in the phage inactivation depicted it to be a heat resistant stationary phase protein on the surface of EcN, which could be a component of its biofilm. In addition, we showed that during coculture, STEC strains can convert harmless *E. coli* K-12 strains into strong stx producers with an increase in toxin level and *stx*-phage titer and this increase can be prevented by the presence of EcN in triculture (EcN, *E. coli* K-12, *stx*-phages).

Conclusions: Our results revealed the complete *stx*-phage resistance of EcN and demonstrated the resistance to be connected to the presence of an intact lambdoid prophage which interferes with superinfection. We unraveled that EcN cannot only effectively reduced the *stx*-phage titer but also interfered with the *stx*-phage infection of *E. coli* K-12 strains. Our findings showed for the first time a phage neutralizing effect by a probiotic strain.

ECMIS
2-5 June 2019, Ghent, Belgium

Intramuscular vaccination with CssBA, a CS6-subunit vaccine candidate against enterotoxigenic *E. coli* (ETEC), and LT(R192G/L211A) as adjuvant promotes antigen-specific α4β7⁺ antibody-secreting cells

Milton Maciel Jr.[1,2], *Stefanie Trop*[1,2], *Aaron Kim*[1,2], *Elizabeth Ward*[1,2], *Zuzana Villar*[1,2], *Steven Poole*[1,2], *Chad Porter*[2], *Tida Lee*[2], *Michael G. Prouty*[2].

[1] Henry M. Jackson Foundation, Bethesda, Maryland, USA
[2] Naval Medical Research Center, Silver Spring, Maryland, USA

Introduction: CssBA, a subunit-based vaccine against ETEC CS6 colonization factor was evaluated in a phase 1 clinical trial with LT(R192G/L211A) (dmLT), an attenuated mutant of the ETEC heat-labile toxin as an adjuvant. Volunteers (n=5-10/cohort) were vaccinated intramuscularly with increasing amounts of CssBA and/or dmLT, as follows: 5 μg CssBA or 0.1 μg dmLT (cohort A), 5 μg CssBA+0.1 μg dmLT (B), 5 μg CssBA+0.5 μg dmLT (C), 15 μg CssBA+0.5 μg dmLT (D), and 45 μg CssBA+0.5 μg dmLT (E).

Aims: Quantify Ag-specific serum IgG and IgA Ab levels as well as α4β7⁺ Ab-secreting cell (ASC) responses promoted by vaccination.

Results: CssBA alone induced 40% (2/5) anti-CS6 IgG serologic responders. Addition of 0.1 or 0.5 μg dmLT increased that to 88% (7/8) and 100% (9/9), respectively, while 45 μg CssBA + 0.5 μg dmLT led to a more rapid response with 100% seroconversion after two vaccinations. Serum anti-CS6 IgA responses were modest and responders only observed in cohorts that received 0.5 μg dmLT (C: 67%; D and E: 50%). High (75-100%) anti-LT IgG responder rates were seen in all cohorts receiving dmLT. In comparison, lower response rates were observed for serum anti-LT IgA (20-70%). Curiously, the highest anti-LT IgG and IgA Ab levels were observed with 5 μg CssBA+0.5 μg dmLT (cohort C). α4β7⁺ CS6-specific IgA and IgG ASC seemed to peak after the third vaccination, regardless of CssBA or dmLT doses, while α4β7⁺ LT-specific ASC responses usually peaked after the second vaccination. 100% α4β7⁺ CS6- and LT-specific IgA and IgG ASC responders were seen in cohort E.

Conclusions: dmLT enhanced IgG and IgA Ab responses against intramuscularly administered CssBA and elicited anti-toxin responses. ELISPOT assays with α4β7⁺ purified cells suggest that vaccination activated plasmocytes with mucosal homing capability. Analyses of antibody in lymphocyte supernatant (ALS) and stool samples are ongoing.

ECMIS
2-5 June 2019, Ghent, Belgium

Oxygen and contact with human intestinal epithelium independently stimulate virulence gene expression in enteroaggregative *Escherichia coli*

*Samuel J. Ellis[1,2], Muhammad Yasir[2,3], Douglas F. Browning[3], Stephen J. W. Busby[3], and Stephanie Schüller[1,2]**

Norwich Medical School, University of East Anglia, Norwich, UK[1],
Quadram Institute Bioscience, Norwich, UK[2],
Institute of Microbiology and Infection, University of Birmingham, Birmingham, UK[3]
s.schuller@uea.ac.uk

Introduction: Enteroaggregative *E. coli* (EAEC) are important intestinal pathogens causing acute and persistent diarrhoeal illness worldwide. Although many putative EAEC virulence factors have been identified, their association with pathogenesis remains unclear.

Aims: As environmental cues can modulate bacterial virulence, we investigated the effect of oxygen and human intestinal epithelium on EAEC virulence gene expression to determine the involvement of respective gene products in intestinal colonisation and pathogenesis.

Results: Using in vitro organ culture of human intestinal biopsies, we established the colonic epithelium as the major colonisation site of EAEC strains 042 and 17-2. We subsequently optimised a vertical diffusion chamber system with polarised T84 colon carcinoma cells for EAEC infection and showed that oxygen induced expression of the global regulator AggR, aggregative adherence fimbriae, *E. coli* common pilus, EAST-1 toxin and dispersin in EAEC strain 042 but not in 17-2. Furthermore, the presence of T84 epithelia stimulated additional expression of the mucinase Pic and the toxins HlyE and Pet. This induction was dependent on physical host cell contact and did not require AggR.

Conclusion: Overall, these findings suggest that EAEC virulence in the human gut is modulated by environmental signals including oxygen and the intestinal epithelium.

ECMIS
2-5 June 2019, Ghent, Belgium

The effects of β-1,3-1,6 glucans on innate immune responses in pigs

Hermans L., Devriendt B., Favoreel H., Cox E.

Laboratory of Immunology, Faculty of Veterinary Medicine

Introduction: Post-weaning diarrhoea caused by enterotoxigenic E. coli (ETEC) is a major problem in swine industry worldwide. It leads to sudden death of piglets, dehydration and growth retardation, resulting in serious economic losses. Since vaccination gives varying results, the prophylactic use of antibiotics is prohibited in Europe and a ban on zinc oxide is foreseen, alternative treatments are needed. Previously our group showed that beta-glucans in feed can protect against an ETEC infection, but the mechanism of action is not known.

Aims: The aims of this study were to investigate the effects of beta-glucans on innate immune responses in pigs in general, and more specifically their interactions with and effects on NK cells.

Results: No direct effects of beta-glucans on the cytotoxic capacity of porcine NK cells were observed. However, when PBMC were stimulated with beta-glucans, NK cell cytotoxicity did increase significantly. When NK cells are removed from the assay, the depleted PBMC population does not show this increase in killed target cells upon priming with beta-glucans. To understand the mechanisms behind the activation of NK cells by glucans through PBMC, cytolytic assays with supernatant of beta-glucan-stimulated PBMC were performed. When added to sorted NK cells, the supernatant was able to stimulate NK cells, leading to increased killing of NK-susceptible target cells. The cells responsible for this indirect activation of NK cells after beta-glucan priming and the factors that they release into the supernatant are not yet identified.

Conclusions: These results show that beta-glucans can indirectly stimulate NK cell cytotoxicity, probably by inducing cytokine production in PBMC. A next step is to identify these soluble molecules and to examine if they might play a role in the activation of other pathways of the innate immune system.

ECMIS
2-5 June 2019, Ghent, Belgium

Improved weight gain and reduced mortality and antibiotic use following an oral vaccination with Coliprotec® F4 in piglets with post-weaning diarrhea

Vangroenweghe Frédéric

Elanco Animal Health, Antwerpen, België

Introduction: Post-weaning *Escherichia coli* diarrhea (PWD) is due to enterotoxigenic *Escherichia coli* (ETEC). Therapy to control PWD typically consists of antibiotic treatment, addition of zinc oxide (ZnO) and/or changes in feed composition.

Aims: The objective of the present study was to compare a vaccination with Coliprotec® F4 to two standard therapeutic approaches, namely ZnO and a safe feed formulation (30% barley) in combination with individual antibiotic treatment in a herd with F4-ETEC PWD. Piglets from a 600-sow farm with F4-ETEC PWD were divided in 5 groups and groups A-B-C vaccinated at 18 days of age with Coliprotec® F4. At weaning, piglets (n=640) in the different groups were attributed to different treatments: (A) 1-phase diet (0-50d) + vaccination; (B) 2-phase diet (0-7d, 8-50d) + vaccination; (C) 3-phase diet (0-10d, 11-25d, 26-50d) + vaccination; (D) 30% barley formulation without antibiotic; (E) ZnO. During the 7-weeks post-weaning period, several production parameters were recorded.

Results: The ZnO group (E) had a significantly better fecal score and a lower treatment incidence compared to groups A-B-C. Compared to group D , the vaccinated piglets (A-B-C) had a significantly higher average daily weight gain, a better feed consumption, a lower mortality and a higher overall financial ROI (€ +5.31). The performance parameters did not differ among the types of diet (1-, 2- or 3-phase diet). The 30% barley group (D) showed the highest mortality in combination with lowest average daily growth and very high antibiotic treatment incidence.

Conclusions: This comparative study clearly shows that a vaccination against PWD with Coliprotec® F4 improves the performance (average daily weight gain) during the post-weaning period. In conclusion, control of PWD through oral vaccination is a good option to protect piglets from the negative effects of a F4-ETEC infection in the post-weaning period.

PART III: SELECTED POSTER PRESENTATIONS

P1. Application of high energy diets in combination with the Coliprotec® F4 vaccination against post-weaning diarrhea

Vangroenweghe Frédéric

Elanco Animal Health, Antwerpen, België

Introduction: Post-weaning *Escherichia coli* diarrhea (PWD) is due to enterotoxigenic *Escherichia coli* (ETEC). Therapy to control PWD typically consists of antibiotic treatment, addition of zinc oxide (ZnO) and/or changes in feed composition.

Aims: The objective of the study was to compare a vaccination with Coliprotec® F4 vaccine in piglets fed different diets (high energy (HE) diet (2530 kcal/kg) with or without acid addition, diet containing ZnO or 30% barley diet). Piglets from a 600-sow farm with F4-ETEC PWD were divided in 5 groups and groups C-D-E vaccinated at 18 days of age with Coliprotec® F4. At weaning, piglets (n=640) were attributed to different treatments: (A) ZnO; (B) 30% barley; (C) 30% barley followed by HE diet + vaccination; (D) HE diet + vaccination; (E) HE diet + acid + vaccination. During the post-weaning period, several production parameters were recorded.

Results: No significant differences were observed among vaccinated groups. In the first 3 weeks post-weaning, the vaccination with Coliprotec® F4 (C-D-E) resulted in a statistically better ($P<0.05$) fecal score (56 AUC3wk), a higher average daily weight gain (150 g/d) and a lower antibiotic treatment incidence (15 TI100) when compared to the piglets fed with the 30% barley formulation (116 AUC3wk, 124 g/d, 52 TI100) (B). Vaccination with Coliprotec® F4 (C-D-E) also resulted in a statistically lower ($P<0.05$) antibiotic treatment incidence (15 TI100) when compared to the piglets on ZnO (25 TI100) (A).

Conclusions: This comparative study shows that a vaccination against PWD with Coliprotec® F4 improves the performance of piglets during the first three weeks of the post-weaning period. The use of HE diets in combination with Coliprotec® F4 resulted in better growth with lower antibiotic use. In conclusion, control of PWD through oral vaccination is a good option to protect piglets from the negative clinical effects of a F4-ETEC infection in the post-weaning period.

ECMIS
2-5 June 2019, Ghent, Belgium

P2. E. coli F4 and F18 vaccination for the prevention of F18-ETEC post-weaning diarrhea

Vangroenweghe Frédéric [1], Crul Stefanie [2], Bonny Peter [2]

1 Elanco Animal Health, Antwerpen, België; 2 DAP Bonny, Beselare, België

Introduction: Post-weaning *Escherichia coli* diarrhea (PWD) in pigs remains a major cause of economic losses for the pig industry. PWD typically causes mild to severe watery diarrhea between 5 and 10 days after weaning, caused by enterotoxigenic *E. coli* (ETEC). Most common adhesins on ETEC from PWD are the fimbriae F4 and F18, while predominant enterotoxins are heat-labile toxin (LT), heat-stable toxin a (STa), and heat-stable toxin b (STb). Therapy to combat PWD typically consists of antibiotic treatment or high doses of ZnO (3000 ppm). An oral live bivalent *E. coli* F4/F18 vaccine (Coliprotec® F4/F18; Prevtec Microbia) is available on the market in Europe, which reduces the impact of PWD provoked by F4- and F18-ETEC.

Aims: The objective of study was to compare the performance of piglets vaccinated against *E. coli* F4/F18 with the ones of piglets treated with the previous standard therapeutic approach (colistin) under field conditions. A 600-sow farm with diagnosed problems of PWD due to F18-ETEC was selected. Piglets were vaccinated at 18 days with the oral live bivalent *E. coli* F4/ F18 vaccine. At weaning, no standard group medication was applied for prevention of PWD. Piglets were fed a farm-prepared liquid feed formula. Performance parameters were collected: weight gain during d0-50, time in nursery, feed conversion ratio, feed cost, mortality, average daily weight gain and medication use (TI100).

Results: Performance parameters were similar before and after the switch to the oral *E. coli* F4/F18 vaccination. However, mortality rate was significantly reduced ($P<0.05$) and the TI100 decreased with 75%.

Conclusions: The results show that vaccination against PWD obtained similar technical performance parameters, in combination with a significant reduction in the medication use. In conclusion, control of PWD through vaccination under field conditions is a good option to protect piglets from the negative clinical effects of a F18-ETEC infection during the post-weaning period.

ECMIS
2-5 June 2019, Ghent, Belgium

P3. Prevalence of virulence factors of *Escherichia coli* isolated from piglets with post-weaning diarrhoea in Belgium and The Netherlands

Vangroenweghe Frédéric [1], Luppi Andrea [2]

1 Elanco Animal Health, Antwerpen, België; 2 IZSLER, Emilia Romagna, Italië

Introduction: Post-weaning *Escherichia coli* diarrhea (PWD) remains a major cause of economic losses for the pig industry. PWD typically causes mild to severe watery diarrhea between 5 and 10 days after weaning and is caused primarily by entero-toxigenic *Escherichia coli* (ETEC). The most common adhesins found in ETEC from pigs with PWD are fimbriae F4 (previously called K88) and F18, while the predominant enterotoxins are heat-labile toxin (LT), heat-stable toxin a (STa) and heat-stable toxin b (STb). Laboratory diagnostics, including characterization of virulence factors, are essential to understand the role of *E. coli* in PWD outbreaks and to initiate appropriate preventive and control measures such as live oral vaccination.

Aims: The objective of the present study was to determine the prevalence of ETEC subtypes causing PWD in Belgium and The Netherlands. A total of 504 pig herds in the Benelux showing clinical signs of PWD were sampled between January 2014 and December 2016. Rectal swab samples (n=5) from diarrheic pigs were collected and submitted to IZSLER (Brescia, Italy) to test for the presence of virulence factors - adhesins (F4, F5, F6, F18 and F41) and toxins (LT, STa, STb, Stx2e) by PCR.

Results: In total 526 non-hemolytic and 784 hemolytic *E. coli* strains were isolated and subsequently tested by PCR. The overall prevalence of the different ETEC subtypes was as follows, F4-ETEC: 24.4% and F18-ETEC: 19.2%. Taking into account that 5 samples were taken in each herd, the prevalence of the different ETEC subtypes at herd level was as follows: F4-ETEC: 45.8% (n=231 herds) and F18-ETEC: 37.5% (n=189 herds). Besides ETEC, 22 isolates (1.7%) were classified as Shiga toxin-producing *E. coli* (STEC).

Conclusions: This study confirms that fimbria type F4 was slightly more prevalent than F18 among *E. coli* isolates from PWD cases in Belgium and The Netherlands.

ECMIS
2-5 June 2019, Ghent, Belgium

P4. Lactoferrins can inhibit enterotoxigenic *E coli* growth and attachment to intestinal epithelial cells

Dierick M [1]., Devriendt B.[1], Vanrompay D. [2], Cox E [1].

1 Laboratory of Immunology, Faculty of Veterinary Medicine, Ugent
2 Department of Molecular Biotechnology, Faculty of Bioscience Engineering, Ugent

Introduction: Post-weaning enterotoxigenic F4+ and F18+ *E. coli* (ETEC) infections have important implication for piglets by decreasing health and quality of life of the piglets. Besides, there are severe economic losses for the pig industry due to diarrhoea, growth retardation, mortality and elevated use of medications. Extensive use of antibiotics and zinc oxide during the first two weeks after weaning is used to control post-weaning diarrhoea and has most likely contributed to an increased occurrence of antibiotic resistant strains. Therefore alternative treatments are needed.

Aims: To evaluate the direct effect of lactoferrins from different species on bacterial growth, bacterial adhesion to enterocytes and stability of fimbriae, flagellin and enterotoxins. Since differences in effect have been seen between lactoferrins, ovotransferrin and bovine lactoferrin were compared.

Results: Bovine lactoferrin and ovotransferrin, which are known to have serine protease activity, were shown to degrade F18 fimbriae, while bovine lactoferrin was also able to degrade F4 fimbriae. In an adhesion assay, we further showed that both bovine lactoferrin and ovotransferrin decreased the attachment of different F4 fimbriated *E. coli* strain to small intestinal epithelial cells. Here, bovine lactoferrin seemed to be more effective in inhibiting this attachment. Bovine lactoferrin was also able to inhibit the growth of all tested ETEC strains, whereas ovotransferrin failed to inhibit this growth.

Conclusions: These findings suggest that lactoferrin might be used to interfere with ETEC colonization and improve gut health in piglets.

ECMIS
2-5 June 2019, Ghent, Belgium

P5. Enterotoxins secretion levels differ in porcine ETEC strains

Haixiu Wang, Eric Cox, Bert Devriendt

Laboratory of Immunology, Faculty of Veterinary Medicine, UGent, Belgium

Introduction: Enterotoxigenic *Escherichia coli* (ETEC) are an important diarrhea-causing pathogen for humans and farm animals. In piglets, most ETEC infections are caused by strains that produce two major enterotoxins, heat-labile enterotoxin (LT) and heat-stable enterotoxin (ST). However, it is unclear if LT and ST secretion levels differ between porcine ETEC strains.

Aims: Here, we aimed to investigate if LT and ST secretion levels differed between porcine ETEC strains.

Results: A total of 32 porcine ETEC strains, isolated from diarrheic piglets, carried LT and STb genes as shown by PCR analysis, while eight strains carried the STa gene. These strains were assessed for their capacity to produce and secrete LT, STa and STb using a GM1-ganglioside, a competitive ELISA and western blotting, respectively. The production of the enterotoxins by these strains was assessed upon disruption of the outer membrane by polymyxin B treatment. All strains had a similar LT production capacity, however, they greatly differed in their ability to secrete LT into the culture supernatant. Preliminary results indicated that gspS protein was involved in LT secretion capacity. In contrast to LT, we observed no discrepancies between the production and secretion of the heat stable enterotoxins, although their secretion levels varied between the different porcine ETEC strains.

Conclusions: Altogether, our results show that enterotoxin secretion levels vary substantially between porcine ETEC strains. Intriguingly, these strains had similar LT production levels. Further research will unravel the molecular mechanisms behind this phenomenon.

ECMIS
2-5 June 2019, Ghent, Belgium

P6. Glyco-engineered cell line and computational docking studies reveal that ETEC CFA/I fimbriae bind to human Lewis a glycans

Mottram L.F [1], Liu J [2], Chavan G. S [3], Larsson G [2], Holgersson J [2], Svennerholm A.M [1]

[1]The Department of Microbiology and Immunology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden. [2]The Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden. [3]Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden
lynda.mottram@gu.se

Introduction: We have previously reported clinical data to suggest that enterotoxigenic *Escherichia coli* (ETEC) CFA/I and related colonisation factors (CFs), bind to the histo-blood group antigen Lewis a (Le^a). Le^a is ubiquitously expressed on glycan structures in the small intestinal mucosa of young children (less than two years of age) and *FUT2*$^{-/-}$ individuals. Moreover, this Le^a expression could affect human susceptibility to severe ETEC infection. We have therefore developed genetically defined models and methods to study such human-ETEC interactions.

Aims: To improve our understanding of ETEC CFA/I pathogenesis in humans by defining ETEC CFA/I and related CF adhesion to Le^a expressing glycan structures.

Results: We created defined human small intestinal like glycan cell line models by engineering Chinese Hamster Ovary (CHO-K1) cells to express Le^a (i.e. *FUT2*$^{-/-}$ individuals) or Le^b (i.e. *FUT2*$^{+/+}$individuals) determinants on both *N*- and *O*-glycans. We found that CfaB, the major subunit of ETEC CFA/I, as well as four related ETEC CFs (CS1, CS2, CS4 and CS14), bind significantly more to our glycan-engineered CHO-K1 cell-line expressing Le^a, compared to cells carrying Le^b or the CHO-K1 wild-type glycan phenotypes. Using computational docking analysis, we predict up to three amino acids (Glu^{25}, Asn^{27}, Thr^{29}) found in the Ig-like groove region of CfaB and related CFs, could be important for the preferential and higher affinity binding of ETEC CFA/I like CFs to Le^a expressing glycan structures.

Conclusions: A critical first step in microbial pathogenesis is frequently the attachment to host glycan structures. Our findings show that ETEC CFA/I and some related CFs preferentially bind more to glycan structures expressing Le^a. Le^a is commonly expressed in glycans in the small intestinal mucosa of children under two years of age and *FUT2*$^{-/-}$ individuals. Such findings could lead to a better understanding of ETEC pathogenesis in humans, aiding in the development of ETEC therapeutics to protect high risk individuals.

ECMIS
2-5 June 2019, Ghent, Belgium

P7. Development, characterization, and immunological evaluation of parenterally delivered CS6-subunit vaccine candidates against enterotoxigenic *E. coli* (ETEC)

Steven Poole[1,2], *Milton Maciel Jr.*[1,2], *Amritha Ramakrishnan*[3], *Yang Liu*[1,2], *Kathleen Dori*[1,2], *Michael G. Prouty*[2].

[1]Henry M. Jackson Foundation, Bethesda, Maryland, USA; [2] Naval Medical Research Center, Silver Spring, Maryland, USA; [3]Naval Medical Research Unit 6, Lima, Peru

Introduction: Enterotoxigenic *E. coli* (ETEC) is a leading bacterial cause of diarrhea in developing countries and travelers. As part of a multivalent ETEC subunit vaccine strategy for parenteral delivery, we constructed a panel of dimeric fusion proteins based on the CS6 subunits, i.e. CssA and CssB. Candidates were screened by their biochemical and immunological features in BALB/c mice and the down-selected candidate was evaluated in a non-human primate (NHP) challenge model to assess immunogenicity and efficacy against the oral challenge with a CS6$^+$ ETEC strain.

Aims: Development of a protein subunit-based vaccine candidate to protect against CS6$^+$ ETEC.

Results: After biochemical and immunological evaluation of a panel of eight CS6 dimers, we selected the N-terminally deleted fusion of CssB and CssA with the donor strand from CssB (ntd_dsc$_B$CssBA) for evaluation in the *Aotus nancymaee* non-human primate challenge model using B7A, a CS6$^+$ ETEC strain. Intradermal vaccination with ntd_dsc$_B$CssBA plus LT(R192G/L211A) (dmLT) as adjuvant elicited maximal anti-CS6 and anti-heat-labile toxin IgG and IgA serological responses after three immunizations. Moreover, vaccination conferred significant protection from diarrhea (0/7 animals meeting definition of diarrhea) when compared to the negative control group that received PBS (5/8) (efficacy 100%, P=0.03). In the same study, vaccination with CS6 protein (derived from ETEC strain E8775) afforded some protection against ETEC challenge (1/8; efficacy 80%, P=0.12).

Conclusions: We identified ntd_dsc$_B$CssBA, a recombinant fusion of the two subunits comprising CS6 (CssB and CssA), as the ideal formulation for inducing a robust and functional immune response. Furthermore, we show that vaccination with ntd_dsc$_B$CssBA was protective against challenge with a CS6$^+$ ETEC strain. A phase 1 clinical trial evaluating the safety and immunogenicity of ntd_dsc$_B$CssBA, administered intramuscularly with dmLT, has recently been completed with the assessment of the immunological parameters still ongoing (see companion abstracts).

ECMIS
2-5 June 2019, Ghent, Belgium

P8. Antibody-mediated selective targeting of oral vaccines to epithelial CD13

Van der Weken H. , Devriendt B. , Cox E.

Laboratory of immunology, Faculty of veterinary Medicine
B.devriendt@ugent.be

Introduction: Enterotoxigenic Escherichia coli (ETEC) are a major cause of intestinal infections in neonatal and recently weaned piglets, resulting in diarrhea, morbidity and mortality. This causes considerable economical losses in the pig industry. To achieve successful immunity against intestinal pathogens, mucosal immunity must be induced. Oral vaccination holds much promise in this regard but is difficult to achieve. Antigens not only have to cross the epithelial barrier but must also circumvent the default tolerogenic immune response present in the gastro-intestinal tract. A possible strategy to overcome these problems is by selectively delivering vaccine antigens to specific intestinal cell populations. Especially targeting of antigens to the epithelial receptor aminopeptidase N (CD13) looks promising. It has been shown that antibodies targeting CD13 become endocytosed and transcytosed by epithelial cells after binding to this receptor.

Aims: Targeting of recombinant antibody-antigen constructs to CD13 could potentially induce a mucosal immune response against an antigen of interest. For this experiment, recombinant monoclonal antibodies targeting CD13 were fused to the FedF antigen. After transfection to CHO cells, production, stability and binding characteristics were assessed.

Results: A recombinant monoclonal antibody targeting CD13, fused to the FedF antigen was constructed and shown to be stably produced in CHO cells. After purification, antibody stability was confirmed using SDS-PAGE and western blotting. Binding to CD13 was confirmed on CD13-expressing cell lines as well as on intestinal ileum and jejunum of 3-week old piglets. Endocytosis of the recombinant antibodies was confirmed on an APN-expressing cell line.

Conclusions: The recombinant antibody-antigen fusion construct showed stable production and similar binding characteristics compared to the original monoclonal antibody targeting CD13. Since endocytosis was also confirmed, this antibody construct will now be assessed for its ability to induce a protective mucosal immune response against F18+ ETEC.

ECMIS
2-5 June 2019, Ghent, Belgium

P9. Investigating mechanisms of Enterohaemorrhagic *Escherichia coli* O157:H7 manipulation of the bovine cellular immune response

Amany Hassan, David Gally, Tim Connelley, Alexander Corbishley

The Roslin Institute, University of Edinburgh

Introduction: Enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7 can cause haemorrhagic diarrhoea and potentially fatal renal failure in humans. Ruminants are considered the primary reservoir for human infection. Studies investigating the response of cattle to colonization generally focus on humoral immunity, leaving the role of cellular immunity unclear. These bacteria colonise their host by tight attachment to the epithelium, using a type three secretion system to inject a cocktail of effectors into the host cell. Injected effectors manipulate the innate response in several ways to promote bacterial persistence. Transcriptional profiling of responses at the terminal rectum, the primary site of colonisation in cattle, reveals a bias towards a T-helper type 1 response, indicating that cellular immunity may be involved in bacterial clearance. Mathematical modelling also indicates that injected effectors have a reduced human MHC-I epitope density, whilst structural bacterial proteins do not. This implies that host cellular immune responses target injected effectors and have exerted selective pressure on their evolution.

Aims: Investigating EHEC O157:H7 manipulation of MHC-I surface expression of cultured bovine epithelial cells following infection with *E. coli* O157. Detecting the presentation of EHEC O157:H7 injected peptides on the surface of MHC-I during infection.

Results: Initial results demonstrate a decrease in MHC-I surface expression during EHEC O157 colonisation after three hours. The basis of this reduction is being investigated using defined mutants in type three secretion genes.

Conclusions: EHEC O157:H7 demonstrated an ability to downregulate MHC-I provided another finding for the potential role of EHEC O157:H7 to manipulate the cellular immune response during colonisation. This observation will be investigated further using peptide elution study and mass spectrometry to examine the presentation of effector protein peptides and determine whether *E. coli* O157 has evolved to interfere with this process.

ECMIS
2-5 June 2019, Ghent, Belgium

P10. Virulotyping of Shiga toxin-producing (STEC) and enteropathogenic (EPEC) *Escherichia coli* isolated from recto-anal mucosal swabs of young diarrheic and non-diarrheic calves

Habets A[1], Engelen F[2], Devleesschauwer B[3,4], De Zutter L[4], Cox E[2], Duprez J-N[1], Thiry D[1], Mainil J[1]

[1] *Laboratory of Bacteriology, Department of Infectious Diseases, FARAH, ULiège.*
[2] *Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, BE*
[3] *Department of Epidemiology and Public Health, Sciensano, Brussels, BE*
[4] *Department of Veterinary Public Health and Food Safety, Faculty of Veterinary Medicine, Ghent*
jg.mainil@uliege.be

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are food-borne pathogens causing severe disease in humans worldwide, whose most important virulence factor is the production of Shiga toxins (Stx1, Stx2). Additionally, most STEC also possess the *eae* gene encoding for the intimin protein, which is responsible for the formation of the attaching and effacing (A/E) lesions (AE-STEC) and is also typical of enteropathogenic *E. coli* (EPEC). AE-STEC colonize the mucosa of the recto-anal junction of healthy adult cattle, making recto-anal mucosal swabs (RAMS) more sensitive than fecal culture to isolate them. In addition, AE-STEC also colonize the small and large intestines of young calves causing diarrhea. Conversely, the carrier state of non-diarrheic young calves is unknown. Finally, most studies address the most pathogenic O157:H7 AE-STEC serotypes, although several other non-O157:H7 AE-STEC serotypes are becoming increasingly important with regard to human outbreaks.
Aims: The objective of the present study was to evaluate the colonization by and shedding of AE-STEC in young diarrheic and non-diarrheic dairy calves. RAMS from 233 young calves in 3 farms with non-O157:H7 AE-STEC in older animals were overnight enriched in Lauryl-Sulfate broth and tested with a triplex PCR targeting the *eae*, *stx1* and *stx2* genes. Positive broths were subsequently streaked on four selective agar plates. Up to 5 colonies per plate will be picked-up, tested by the colony hybridization assay with probes for the same genes and confirmed by the same triplex PCR.
Results: A total of 152 broths (65%) tested positive with the triplex PCR after overnight enrichment: 23 for AE-STEC, 122 for STEC and 7 for EPEC. In farm #1, 2 RAMS tested positive for AE-STEC, 45 for STEC and 3 for EPEC. In farm #2, 3 RAMS tested positive for AE-STEC, 42 for STEC and 4 for EPEC. In farm #3, 18 RAMS tested positive for AE-STEC and 35 for STEC. All positive broths are being streaked on the selective agar plates and the 5 picked-up colonies are being prepared for the colony hybridization assay.
Conclusions: Of the positive RAMS the majority (85%) harbored STEC and a minority AE-STEC (15%) or EPEC (5%). This low number of EPEC-positive samples is different from previous results obtained in adult cattle in two slaughterhouses following the same methodology. In the same survey, 80% of the PCR-positive broths gave colony hybridization-positive and triplex PCR-positive colonies. Confirmed positive colonies will be O:H serotyped by PCR and all results will be associated with presence/absence of diarrhea in the calves.

ECMIS
2-5 June 2019, Ghent, Belgium

P11. Identification of non-conventional serotypes of enteropathogenic (EPEC) *Escherichia coli* isolated from diarrheic calves

Habets A[1], Duprez J-N[1], Iguchi A[2], Saulmont M[3], Mainil J[1], Thiry D[1]

[1]Bacteriology, Department of Infectious Diseases, FARAH, ULiège.
[2]Department of Animal and Grassland Sciences, Faculty of Agriculture, University of Miyazaki, Japan.
[3]Regional Agency for Animal for Health and Identificatio@n (ARSIA), Ciney, Belgium.
jg.mainil@uliege.be

Introduction: Enteropathogenic *Escherichia coli* (EPEC) are subdivided into "typical (t) EPEC" producing the "Bundle Forming Pili" (BFP) type 4 fimbriae and isolated from humans, and "atypical (a) EPEC" not producing the BFP and isolated from animals and humans. aEPEC are indeed quite frequently associated with diarrhoea in young calves. Although calf aEPEC can belong to the O26:H11 and O80:H2 serotypes, most serotypes remain unidentified.

Aims: The general purpose of this project is to identify the serotypes of aEPEC isolated from diarrheic calves and to compare them with calf and human Shiga toxin-producing *E. coli* (STEC) belonging to the same serotypes.
The specific purpose of the study reported here was therefore to test 41 un-typed aEPEC for five non-conventional O serogroups (O123/186, O156, O177, O182, O183) recently identified in 5 calf aEPEC and STEC that previously tested negative, using two multiplex PCRs and to confirm the positive results with the uniplex PCRs. For comparison 35 STEC also belonging to still unidentified serogroups were included in the study.

Results: Twenty-three aEPEC (56%) tested positive with the multiplex and uniplex PCRs: 9 for the O123/186 serogroups (these two serogroups cannot be distinguished by PCR), 12 for the O177 and 2 for the O182 serogroups. In addition, the PCRs also detected 8 STEC (23%): 1 for the O123/186, 2 for the O156, 1 for the O177, 2 for the O182 and 2 for the O183 serogroups.

Conclusions: Besides the classical O26:H11 serotype, calf aEPEC belong to several non-conventional serogroups/-types, like O80:H2 and those identified in this study, though still other serogroups/-types remain to identify. The further steps are: (i) comparison of these calf aEPEC with calf and human STEC belonging to the same serogroups/-types; (ii) investigation to answer the following question: are these calf aEPEC true aEPEC, or STEC derivatives that have lost *stx* genes, or STEC precursors that could acquire *stx* genes in the future? (iii) identification of still other serogroups/-types amongst the remaining un-typed calf aEPEC.

ECMIS
2-5 June 2019, Ghent, Belgium

P12. Increased production of Shiga toxin by antimicrobial treatment – statement revisited

Silje N Ramstad[1], Arne M. Taxt[1], Umaer Naseer[2], Yngvild Wasteson[3], and Lin T Brandal[2].

[1]Oslo University Hospital
[2]Norwegian Institute of Public Health
[3]Norwegian University of Life Sciences

Introduction: Shiga toxin producing *Escherichia coli* (STEC) can cause bloody diarrhea and hemolytic uremic syndrome (HUS) in humans. Currently, antibiotic treatment of STEC infections is not recommended, as studies indicate that antibiotics induce Shiga toxin (Stx) production and increase the risk of developing HUS. However, recent studies have suggested that some antibiotics might eliminate the pathogen without worsening the disease outcome. These studies have mainly focused on STEC serotypes O157:H7 and O104:H4.

Aims: To investigate the *in vitro* effects of different classes of antibiotics on *stx2a* transcription and Stx production in STEC of different serotypes (NSF O157:H7, O104:H4, O26:H11, O145:H25, SF O157:H7, O103:H25).

Results: We used liquid broth cultures of 12 high-virulent STEC strains of six different serotypes to investigate the effects of sub-minimum inhibitory concentrations (MIC) of six different classes of antibiotics on *stx2a* transcription (quantitative real-time PCR, ΔΔCt method) and Stx production (ELISA). Sub-MIC of gentamicin after two hours induction did not induce *stx2a* transcription in any of the 12 STEC strains analyzed. Doxycycline, meropenem and rifampicin induced *stx2a* transcription in STEC of serotype O104:H4, whereas ciprofloxacin and azithromycin showed weak induction of *stx2a* transcription in STEC of various serotypes. By analyzing the total Stx-amount in the samples after 24 hours we found that azithromycin, gentamicin and meropenem did not induce Stx production in any of the 12 STEC strains examined. Ciprofloxacin increased Stx production in nearly all STEC strains, while doxycycline and rifampicin increased Stx production in some but not all of the strains analyzed.

Conclusions: Our results indicate that some classes of antibiotics have little or no effect on *stx2a* transcription and Stx production in STEC of various serotypes. These results hold promise for antibiotic treatment of STEC infections, but further *in vivo* studies are needed to confirm our findings.

ECMIS
2-5 June 2019, Ghent, Belgium

P13. Innate immune response to EPEC infection in the newborn mouse

Dupont A.[1], *Pütz A.*[1], *Rosinski A.N.*[1], *Hornef M.*[1]

1 Institute for Medical Microbiology, University Hospital RWTH Aachen, Aachen, Germany
adupont@ukaachen.de

Introduction: Enteropathogenic *Escherichia coli* (EPEC) is a major causative agent of infantile diarrhea in developing countries. Due to the absence of a suitable mouse model, EPEC pathogenesis has mostly been studied using *in vitro* models. Recently, we have developed a neonatal mouse model of infection that allows the study of EPEC pathogenesis *in vivo*. In fact, infection of newborn animals with EPEC E2348/69 leads to the formation of intimately attached EPEC microcolonies on the surface of the small intestinal mucosa (A/E lesions).

Aims: To characterize the host innate immune response to EPEC infection *in vivo* using our newly established neonatal mouse model.

Results: Infection of newborn mice with EPEC triggers a T3SS-dependent host immune response, as seen with the increased expression of epithelial *Reg3γ* and *Il-18* 8 days post-infection. In addition, EPEC infection leads to the activation (cleavage) of IL-18 in intestinal epithelial cells, even in the absence of caspase 1, the central component of the classical inflammasome pathway. This host response is abrogated in the absence of hematopoietic MyD88 signalling.

Conclusions: Our data suggest that both sub-epithelial hematopoietic cells and intestinal epithelial cells sense and respond to EPEC infection in a T3SS-dependent manner. In addition, these results highlight the importance to complement *in vitro* studies with more complex *in vivo* models to fully understand the exact pathogenesis of EPEC.

ECMIS
2-5 June 2019, Ghent, Belgium

P14. In vitro inhibition of *Escherichia coli* by supernatant of *Bacillus coagulans* PP01

Kadlec, R.[1], Rysavka, P.[2], Zouharova, M.[3], Gebauer, J.[3], Faldyna, M.[3]

1Dairy Research Institute, Prague, Czech Republic
2 Pharmaceutical Biotechnology, s.r.o., Prague, Czech Republic
3 Veterinary Research Institute, Brno, Czech Republic
faldyna@vri.cz

Introduction: *Escherichia coli* is a part of the intestinal microflora. Due to the formation of adhesive molecules and toxins, some strains can cause intestinal infections in humans and animals, incl. post-weaning diarrhea in piglets. One of the way how to try to manage the problem is to use probiotic culture which is able to inhibit the grow of *Escherichia coli.*

Aims: Aim of the study was to test ability of supernatant of *Bacillus coagulans* PP01 to inhibit *Escherichia coli in vitro*.

Results: Selected strain of *Bacillus coagulans* PP01 was positive for enzymatic activity of leucine-, valine- and cystine-arylaminase, naphthol-AS-BI-physphohydrolase, α-glucosidase, β-glucosidase and N-Acetyl-β-glucosaminidase. Ability of supernatant to inhibit grow of *E.coli* was tested by modification of agar diffuse agar. It was found that the supernatant was able to down-regulate E.coli in a dose-dependent manner. Mean inhibition zone diameter ranged from 0 to 16 from 0.5 to 10 mg of supernatant, respectively.

Conclusions: In the experiment, ability of supernatant of *Bacillus coagulans* PP01 to inhibit *Escherichia coli* grow in vitro was proven. This work was supported by the project QK1810463 of Ministry of Agriculture of the Czech Republic.

ECMIS
2-5 June 2019, Ghent, Belgium

P15. Presence of *Escherichia coli* O26 and O157 in young dairy calves by recto-anal mucosal swab (RAMS) culture

Engelen F.[1], Devleesschauwer B.[2,3], Thiry D.[4], Mainil J.[4], Habets A.[4], Heyndrickx M.[5], De Zutter L.[2], Cox E.[1]

[1]*Laboratory of Immunology, Faculty of Veterinary Medicine, UGent*
[2]*Dept. of Veterinary Public Health and Food Safety, Faculty of Veterinary Medicine, UGent*
[3]*Department of Epidemiology and Public Health, Sciensano, Brussels*
[4]*Laboratory of Bacteriology, Department of Infectious Diseases, FARAH, ULiège.*
[5]*Institute for Agricultural and Fisheries Research, Technology & Food Science Unit, Melle*
frederik.engelen@ugent.be

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) cause hemorrhagic colitis and hemolytic-uremic syndrome in humans. Cattle represent the major natural reservoir. Human infection occurs predominantly through consumption of fecally contaminated foodstuffs. The main virulence factor is the production of Shiga toxins (*stx*). Additionally, most strains possess the *eae* gene, responsible for attaching-effacing (A/E) lesions. AE-STEC colonize the mucosa of the bovine recto-anal junction, as demonstrated by culturing recto-anal mucosal swabs (RAMS). While most studies have addressed O157 STEC, more research should be conducted on non-O157 STEC, such as O26, which are becoming more prevalent in human outbreaks.

Aims & methods: The objective was to investigate *E. coli* O26 and O157 presence in young dairy calves. Three Belgian farms (A, B, C), with confirmed *E. coli* O26 and O157, were visited three times. In total, 233 RAMS were collected: 74 on farm A, 71 on farm B, and 88 on farm C. For O157, RAMS were enriched in mTSBn, followed by immunomagnetic separation. For non-O157, RAMS were enriched in Brila broth, followed by a selective acid treatment. Suspected colonies were analysed for virulence genes (*eae*, *stx*) and O-group by multiplex PCR.

Results: On farm A, 24% (18/74) of RAMS were positive for *E. coli* O26, and 34% (25/74) were positive for *E. coli* O157. On farm B, 52% (37/71) were positive for *E. coli* O26, but all were negative for *E. coli* O157. On farm C, 19% (17/88) were positive for *E. coli* O26, and 23% (20/88) were positive for *E. coli* O157. All *E. coli* O157 isolates showed a predominant *eae/stx2* virulence gene profile, while the *E. coli* O26 isolates had varied virulence gene profiles.

Conclusions: These results demonstrate that young dairy calves are frequently being colonized by *E. coli* O26 and O157, and highlights the fact that young animals play a role in on-farm transmission.

ECMIS
2-5 June 2019, Ghent, Belgium

P16. NOVEL 12-MER PEPTIDE DERIVED FROM CPE30 OF CLOSTRIDIUM PERFRINGENES TARGETS M CELLS

Kien-Quang Huynh[1,+], See-Chuan Yam[2,+], An Nguyen-Hoang[1], Vertika Gautam[2], Shu-Ping Lin[3], Khai-Hoan Nguyen-Phuoc[1], Byeol-Hee Cho[5], Kai-Wei Shih[3], Tung Yen Lai[4], Sharifuddin Md Zain[2], Noorsaadah Abd Rahman[2], Linh-Thuoc Tran[1], Yong-Suk Jang[5,6], Vannajan Sanghiran Lee[2,], Hieu Tran-Van[1,*]*

[1]Department of Molecular and Environment Biotechnology, Faculty of Biology and Biotechnology, University of Science, Vietnam National University-Hochiminh, Vietnam.
[2]Department of Chemistry, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia.
[3]Graduate Institute of Biomedical Engineering, National Chung Hsing University, Taichung 40227, Taiwan, R.O.C.
[4]National Applied Research Laboratories, National Nano Device Laboratories, Hsinchu 30078, Taiwan, R.O.C.
[5]Department of Bioactive Material Sciences, Chonbuk National University, Jeonju 54896, Korea
[6]Department of Molecular Biology, Chonbuk National University, Jeonju 54896, Korea
**tvhieu@hcmus.edu.vn; *vannajan@um.edu.my*
[+]First-authorship shared

Introduction: Oral vaccine has been become the most effective strategy in fighting against intestinal infections because of its ease, economy, and capability on inducing responses not only systemic but also local, especially in mucosal compartments. The major obstruction to potent vaccine development is antigen dispersion and tolerance.

Aims: To overcome this hindrance, we aimed to target M cells, which are main sentinel gateway for taking up luminal antigens and initiating specific mucosal responses.

Results: In this study, 12-mer peptide (CPE12) derived from 30 amino acids (CPE30) of *Clostridium perfringenes* was predicted by bioinformatic tools. To confirm the interaction between CPE12 and M cell receptor, the coding gene for CPE12 was cloned, expressed, purified, and evaluated in vitro and ex vivo afterwards. As a result, via silicon nanowire field-effect transistors chip (SiNW FET), CPE12 showed interaction with Claudin-4 with lower extent than that of CPE30. In line with SiNW FET result, ex vivo assessment on murine M cells demonstrated that CPE12 had lower binding on murine M cells surface comparing to CPE30.

Conclusions: These present results suggest that CPE12 could be a competent candidate peptide for oral vaccine development

ECMIS
2-5 June 2019, Ghent, Belgium

LIST OF ATTENDEES

Meryem Aloulou
CRCN, INSERM, Center for Pathophysiology of Toulouse Purpan, Toulouse University, France

Frederic Auvray
Institut de Recherche en Santé Digestive (IRSD) : UMR INSERM 1220, INRA 1416, ENVT, UT3, Toulouse, France

Shahida Baqar
NIH / NIAID, USA

Katja Brunner
Institut Pasteur, France

Eric Cox
Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium

Henri De Greve
Vrije Universiteit Brussel, Belgium

Dereu André
Qui-Vive bvba, Belgium

Bert Devriendt
Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium

Matthias Dierick
Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium

Qiangde Duan
Yangzhou University, China

Silke Dubbert
Pharma-Zentrale GmbH, Germany

Edward G. Dudley
The Pennsylvania State University, and Director of *E. coli* Reference Center, University Park, PA 16802, USA

Aline Dupont
University Hospital Aachen, Germany

Frederik Engelen
Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium

Martin Faldyna
Veterinary Research Institute, Czech Republic

James M. Fleckenstein
Infectious Disease Division, Washington University School Of Medicine, Missouri, USA

Irene Galé
Hipra, Spain

Stephanie Ganal-Vonarburg
Division of Gastroenterology, Department for BioMedical Research (DBMR), University Clinic for Visceral Surgery and Medicine, University of Bern, 3008 Bern, Switzerland

Nichole Giller
Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, Enteric Diseases Department, Naval Medical Research Center, Silver Spring, MD, USA

Bruno Goddeeris
Katholieke Universiteit Leuven, Belgium

Henk Haagsman
Department of Infectious Diseases & Immunology, Veterinary Medicine, Utrecht University Utrecht, The Netherlands

Eric Hall
Naval Medical Research Center, Silver Spring, MD, USA

Kurt Hanevik
Department of Clinical Science, Faculty of Medicine, University of Bergen, Bergen, Norway

Amany Hassan
The Roslin Institute, University of Edinburgh, United Kingdom

Leen Hermans
Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium

Marc Heyndrickx
ILVO, Belgium

Catherine Ionescu
Pancosma, Switzerland

Traute Janβen
RIPAC-LABOR GmbH, Germany

Alireza Khadem
Innovad, Belgium

Anne Kijewski
Norwegian Institute of Life Sciences, Norway

Helle D. Kjaersgaard
Orionpharma Animal Health, Denmark

Birgit Klinkert
Ardeypharm GmbH, Germany

Astrid Koppenol
Impextraco NV, Belgium

Renee Laird
Enteric Diseases Department, Naval Medical Research Center, Silver Spring, MD, USA

Tida Kumbalasiri Lee
Enteric Diseases Department, Naval Medical Research Center, Silver Spring, MD, USA

Alexia Lepine
Orffa Additives B.V., The Netherlands

Kirsten Leurs
Ghent University, Belgium

Emile Libbrecht
IDT Biologika, Belgium

Milton Maciel, Jr.
Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, Enteric Diseases Department, Naval Medical Research Center, Silver Spring, MD, USA

Jacques Mainil
University of Liège, Belgium

Joaquim Mallorqui
Hipra Scientific, Spain

Lynda Mottram
University of Gothenburg, Sweden

Katerina Nechvatalova
Veterinary Research Institute, Czech Republic

Hilde Nelis
Federal Agency of Medicines and Health Products (FAGG), Belgium

Tobias Ölschläger
University of Wuerzburg, Germany

Michaël Pelst
Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium

Marco Poncelas
Hipra, Spain

Steven Poole
Henry M. Jackson Foundation for the Advancement of Military Medicine, Silver Spring, MD, USA

Toni Prenafeta
Hipra, Spain

Michael Prouty
Enteric Diseases Department, Naval Medical Research Center, Silver Spring, MD, USA

Md Mizanur Rahman
Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium

Silje Ramstad
Oslo University Hospital, Norway

Amable Rivas
Vaxxinova, Germany

Xavier Saelens
VIB & Ghent University, Belgium

Christina Schäffer
Department für NanoBiotechnologie, NanoGlycobiology unit, BOKU, University of Natural Resources and Life Sciences, Austria

Stephanie Schüller
University of University of East Anglia, UK

Benedikte Serruys
Ablynx, a Sanofi company, Belgium

Åsa Schöling
Department of Microbiology, Tumor and Cell Biology (MTC), Karolinska Institutet, Stockholm, Sweden

Manonmani Soundararajan
Institute for Molecular Infection Biology, Wuerzburg, Germany

Daniel Sperling
Ceva Sante Animale, Czech Republic

Ann-Mari Svennerholm
Department of Microbiology and Immunology, University of Gothenburg, Sweden

Makrina Totsika
Queensland University of Technology, Australia

Hieu Tran-Van
Faculty of Biology and Biotechnology, University of Science, VNU-HCM, Vietnam

Hans Van der Weken
Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium

Hugo Van Heuverswyn
Flanders Vaccine C/O Biomaric, Belgium

Tim Vandecasteele
Proviron Industries nv, Belgium

Erik Vanderbeke
AVEVE, Belgium

Frédéric Vangroenweghe
Elanco Animal Health, Belgium

Daisy Vanrompay
Molecular Biotechnology, Faculty of Bioscience Engineering, Ghent University, Belgium

Eva Vávrov
Elanco Animal Health, Czech Republic

Bjarne Vermeire
Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium

Vikram Virdi
Department of Plant Systems Biology, VIB (Life Sciences Research Institute), Ghent, Belgium

Darya Vodolazska
Aarhus University, Denmark

Rudolf von Bünau
Pharma-Zentrale GmbH, Germany

Haixu Wang
Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium

Eveline Weerdenburg
Janssen Vaccines & Prevention, The Netherlands

Weiping Zhang
Department of Pathobiology, University of Illinois at Urbana-Champaign, Illinois, USA

Matthew Zhou
GenScript Biotech B.V., The Netherlands

Guoqiang Zhu
Yangzhou University, China

ECMIS
2-5 June 2019, Ghent, Belgium

LIST OF INVITED SPEAKERS

Meryem Aloulou
CRCN, INSERM, Center for Pathophysiology of Toulouse Purpan, Toulouse University, France

Frederic Auvray
Institut de Recherche en Santé Digestive (IRSD) : UMR INSERM 1220, INRA 1416, ENVT, UT3, Toulouse, France

Katja Brunner
Institut Pasteur, France

Edward G. Dudley
The Pennsylvania State University, and Director of *E. coli* Reference Center, University Park, PA 16802. USA

James M. Fleckenstein
Infectious Disease Division, Washington University School Of Medicine, Missouri, USA

Stephanie Ganal-Vonarburg
Division of Gastroenterology, Department for BioMedical Research (DBMR), University Clinic for Visceral Surgery and Medicine, University of Bern, 3008 Bern, Switzerland

Henk Haagsman
Department of Infectious Diseases & Immunology, Veterinary Medicine, Utrecht University Utrecht, The Netherlands

Christina Schäffer
Department für NanoBiotechnologie, NanoGlycobiology unit, BOKU, University of Natural Resources and Life Sciences, A-1190 Vienna, Austria

Åsa Schöling
Department of Microbiology, Tumor and Cell Biology (MTC), Karolinska Institutet, Stockholm, Sweden

Ann-Mari Svennerholm
Department of Microbiology and Immunology, University of Gothenburg, Sweden

Vikram Virdi
Department of Plant Systems Biology, VIB (Life Sciences Research Institute), Ghent, Belgium

Weiping Zhang
Department of Pathobiology, University of Illinois at Urbana-Champaign, Illinois, USA