noro2012

Norovirus and Other Caliciviruses on the Rise

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Organisation and Imprint

Venue and Date
ATLANTIC Hotel Lübeck
Schmiedestraße 9-15
23552 Lübeck, Germany

March 20-22, 2012

Conference Website
www.noro2012.com

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Jacques Rohayem (Riboxx, Radebeul/Dresden, Germany)
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CONGRESS • EVENTS • INCENTIVES
SYMPOSIS

Acknowledgement
We thank Dr. Qingjun Ma, University of Lübeck, for the norovirus image that we used for the logo of noro2012. This image was generated using atomic coordinates in Protein Data Bank entry 1IHM (B.V. Prasad et al.: *Science* **286**, 287-290 (1999)).

Print
SatzPartner
Dienstleistungen rund um den Druck

Kanalstraße 70
23552 Lübeck, Germany
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WELCOME

Dear colleagues,

It is our great pleasure to welcome you to the conference "noro2012: Norovirus and Other Caliciviruses on the Rise" in the historic city of Lübeck. With this scientific meeting, we are trying new ways in promoting research on caliciviruses, as the conference is exclusively focused on this subject. We thought that the continuous rise in numbers of norovirus outbreaks over the past ten years justifies such a focus, rather than having caliciviruses only as one subject among many at the general virology meetings.

The number of registered participants (just below 120) and the submission of more than 65 exciting abstracts certainly support our idea. We will have interesting talks on calicivirus epidemiology, several case reports on norovirus outbreaks, and a strong collection of presentations on norovirus contamination in food and drinking water. The epidemiological aspects will relate to recent insight in calicivirus evolution, and the latter subject will also be illuminated in terms of receptor usage. Due to their obvious importance, we will have several presentations on the atomic details of virus-receptor interactions, and it is here, where structural biology provides important knowledge, along of course with the three-dimensional structures that have been determined for entire virus-like particles and capsid proteins as well as non-structural proteins of caliciviruses. No need to mention that these studies also provide the basis for the rational design of antivirals, an area that is just beginning to be explored. Furthermore, sessions on immunology and vaccine development as well as on basic molecular virology will be essential parts of the programme.

A special session will be devoted to the discussion of murine norovirus as a trigger for M. Crohn-like disease in mice, and this will also include aspects of the interaction triad between the host, the microbiota, and the virome. Finally, approaches to establishing new animal models and norovirus-infected cell cultures will be described.

We are very grateful to all of you for coming to Lübeck and for making important scientific contributions. We thank the invited speakers, all of whom accepted our invitation with great enthusiasm, and our exhibitors and sponsors, whose support made the conference financially viable. We hope that the meeting will be both scientifically beneficial and personally pleasing for you. Enjoy the coming 2 1/2 days in Lübeck!

Rolf Hilgenfeld

Thomas Peters

Norbert Tautz

Gregor Meyers

Jacques Rohayem

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Conference Programme

Tuesday, March 20, 2012

09:00 - 09:15 ***Conference Opening***
Thomas Peters (Lübeck, Germany)

Session 1: Epidemiology, Diagnostics, and Clinical Aspects

Chair (part I): **Ralph Baric** (Chapel Hill, NC, USA)

09:15 - 09:45 **O1.1 – Marina Höhne** (Berlin, Germany):
Detection and Epidemiological Surveillence of Norovirus Infections in Germany during the Past Ten Norovirus Seasons

09:45 - 10:05 **O1.2 – James Ayukekbong** (Gothenburg, Sweden):
Prevalence and Genetic Diversity of Noroviruses in Healthy School Children in Cameroon

10:05 - 10:25 **O1.3 – Nancy P. Nenonen** (Gothenburg, Sweden):
Comparison of Two Norovirus Outbreaks in the Gothenburg Region

10:25 - 10:45 **O1.4 – Julianne R. Lockwood** (London, UK):
Development of a Next Generation Sequencing Pipeline for Norovirus from Clinical Samples

10:45 - 11:15 Coffee Break

Chair (part II): **Stefan Taube** (Ann Arbor, MI, USA)

11:15 - 11:35 **O1.5 – Faizel H.A. Sukhrie** (Bilthoven, The Netherlands):
P2 Domain Profiles and Shedding Dynamics in Prospectively Monitored Norovirus Outbreaks

11:35 - 11:55 **O1.6 – Mathias F.C. Beersma** (Rotterdam, The Netherlands):
Descriptive Analysis of a Cohort of Chronic NoV Patients (n=40) in a Tertiary Care Hospital Setting (2003-2011)

11:55 - 12:15 **O1.7 – Maren Eggers** (Stuttgart, Germany):
A New Method for the Evaluation of Hand Disinfectants against Norovirus Simulating Practical Conditions: The Modified EN 1500 Test

12:15 - 13:30 Lunch

Session 2: Immunology and Vaccine Development

Chair: **Ian Clarke** (Southampton, UK)

13:30 - 14:00 **O2.1 – Ralph Baric** (Chapel Hill, NC, USA):
Cruising with Noroviruses: Mechanisms of Immune Evasion

14:00 - 14:30 **O2.2 – Xi Jiang** (Cincinnati, OH, USA):
Challenges and Solutions on Vaccine Development against Noroviruses

14:30 - 14:55 **O2.3 – Alexander J. Chettle** (Cambridge, UK):
The Role of Type I Interferon in Persistent Murine Norovirus Infection

14:55 - 15:15 **O2.4 – Marc Ehlers** (Lübeck, Germany):
Influence of Vaccination Strategies on IgG Fc Glycosylation

15:15 - 15:45 Coffee Break

Session 3: Virus-Receptor Interactions

Chair: **Ian Goodfellow** (London, UK)

15:45 - 16:10 **O3.1 – Ming Tan** (Cincinnati, OH, USA):
Conservation and Flexibility of the HBGA-Binding Interfaces of Noroviruses: Two Essential Strategies of Norovirus Survival in Human Hosts

16:10 - 16:40 **O3.2 – Göran Larson** (Gothenburg, Sweden):
Studies of Norovirus Interactions with Glycoconjugates Using Virus-like Particles: What Is Happening over Time?

16:40 - 17:10 **O3.3 – Thomas Peters** (Lübeck, Germany):
Insights into Norovirus-Carbohydrate Interactions at Atomic Resolution from NMR

17:10 - 17:35 **O3.4 – Stefan Taube** (Ann Arbor, MI, USA):
Murine Noroviruses Bind Glycolipid and Glycoprotein Attachment Receptors in a Strain-Dependent Manner

17:35 - 18:00 **O3.5 – Marta Bally** (Gothenburg, Sweden):
Recognition of Cell-Membrane Mimics Containing Glycosphingolipids by Norovirus GII.4 Virus-like Particles

18:00 - 19:30 Get-together, Posters & Exhibition

19:30 "Historic Lübeck at Night" (Guided Tour)

Wednesday, March 21, 2012

Session 4: Food Safety

Chair: **Marina Höhne** (Berlin, Germany)

09:00 - 09:20 **O4.1 – James Lowther** (Weymouth, UK):
A Two-Year Systematic Study to Assess Norovirus Contamination in UK Oysters

09:20 - 09:40 **O4.2 – Leena Maunula** (Helsinki, Finland):
Identification of Noroviruses in Food – Finnish Experiences

09:40 - 10:00 **O4.3 – Katharina Verhaelen** (Bilthoven, The Netherlands):
Persistence of Human Norovirus GII.4 and GI.4, Murine Norovirus, and Human Adenovirus on Soft Berries as Compared with PBS at Commonly Applied Storage Conditions

10:00 - 10:20 **O4.4 – Anna Charlotte Schultz** (Søborg, Denmark):
Detection and Quantification of Norovirus in Raspberries Implicated in Disease Outbreaks

Session 5: Structural Biology and Antiviral Drug Discovery

Chair: **Xi Jiang** (Cincinnati, OH, USA)

10:20 - 10:50 **O5.1 – Bidadi V. Prasad** (Houston, TX, USA):
Structural Biology of Caliciviruses

10:50 - 11:20 Coffee Break

11:20 - 11:50 **O5.2 – Martino Bolognesi** (Milan, Italy):
Structure-Based Inhibitors of Norovirus RNA-Dependent RNA Polymerases

11:50 - 12:15 **O5.3 – Surender Vashist** (London, UK):
RNA-Protein Interaction Networks Required for Norovirus Replication: Towards development of Antivirals

12:15 - 13:30 Lunch

Session 6: Molecular Biology of Norovirus

Chair: **Jacques Le Pendu** (Nantes, France)

13:30 - 14:00 **O6.1 – Ian Clarke** (Southampton, UK):
Norovirus Chimaeras: Are They Useful for Studying Gene Function?

14:00 - 14:30 **O6.2 – Ian Goodfellow** (London, UK):
Using Proteomics, Reverse Genetics, and Animal Models to Understand Noroviruses

14:30 - 14:55 **O6.3 – Christine Luttermann** (Greifswald - Insel Riems, Germany):
Differences in AUG Recognition for the Termination Reinitiation Mechanism of Different Caliciviruses

14:55 - 15:20 **O6.4 – Annelies Kroneman** (Bilthoven, The Netherlands):
Unified Proposal for Norovirus Genotyping and Nomenclature

15:20 - 15:50 Coffee Break

Session 7: Pathogen-Host Interactions in Crohn's Disease

Chair: **Martino Bolognesi** (Milan, Italy)

15:50 - 16:20 **O7.1 – Larissa Thackray** (St. Louis, MO, USA):
Intersection of Murine Norovirus with the Atg5-Atg12/Atg16L1 Autophagy Complex

16:20 - 16:50 **O7.2 – Philip Rosenstiel** (Kiel, Germany):
Host-Microbiome-Virome Interactions in the Intestinal Tract: IBD and Beyond

16:50 - 17:15 **O7.3 – John F. Baines** (Plön, Germany):
Colonic Mucosa-Associated Microbiota Is Influenced by an Interaction of Crohn's Disease and FUT2 (Secretor) Genotype

17:15 - 18:00 Posters & Exhibition

18:30 Reception by the Mayor of Lübeck in the Historic Town Hall

19:30 Conference Dinner in the "Ratskeller"

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Thursday, March 22, 2012

Session 8: Other Caliciviruses

Chair: **Bidadi V. Prasad** (Houston, TX, USA)

09:00 - 09:30 **O8.1 – Gregor Meyers** (Greifswald - Insel Riems, Germany):
Cis-acting RNA Structures Promoting Translation Reinitiation in Caliciviruses

09:30 - 09:50 **O8.2 – Peter H. Otto** (Jena, Germany):
Infection of Calves with Bovine Norovirus GIII.1 Strain Jena Virus: An Experimental Model to Study the Pathogenesis of Norovirus Infection

09:50 - 10:10 **O8.3 – Elisabeth M. Liebler-Tenorio** (Jena, Germany):
Infection of Calves with Bovine Norovirus GIII.1 Strain Jena Virus: Intestinal Lesions and Distribution of Viral Antigen

Session 9: Calicivirus Evolution and Epidemiology

Chair: **Göran Larson** (Gothenburg, Sweden)

10:10 - 10:40 **O9.1 – Jacques Le Pendu** (Nantes, France):
Potential Involvement of Histo-Blood Group Antigens in Host-Calicivirus Co-Evolution

10:40 - 11:10 Coffee Break

11:10 - 11:40 **O9.2 – Marion Koopmans** (Bilthoven, The Netherlands):
Lessons from Integrated Molecular and Epidemiological Surveillance of Noroviruses in an International Context

11:40 - 12:05 **O9.3 – Dieter Hoffmann** (München, Germany):
Intraindividual Noroviral Evolution Analyzed by Next Generation Sequencing

12:05 - 12:30 **O9.4 – David J. Allen** (London, UK):
Molecular Evolution of GII-4 NoV Strains

12:30 ***Closure of Conference***
Gregor Meyers (Greifswald - Insel Riems, Germany)
Conclusions

12:40 - 14:00 Lunch

Abstracts for Oral Presentation

Session 1 – Epidemiology, Diagnostics, and Clinical Aspects

O1.1 – Detection and Epidemiological Surveillance of Norovirus Infections in Germany during the Past Ten Norovirus Seasons

Marina Höhne

Consultant Laboratory for Noroviruses, Robert Koch Institute, Berlin, Germany

Human noroviruses are now recognized as the most frequent cause of outbreaks of gastroenteritis worldwide. Although, the infection is usually moderate, it can be especially virulent in young children and the elderly, and constitutes significant economic und health burdens. Surveillance and outbreak investigations are crucial for the understanding of disease frequency, patterns of spread of infection, risk factors for infection and use of intervention and control measures. In recent years, advances in molecular techniques vastly improved the knowledge of strain diversity and emergence of new variants and led to the refinement of diagnostic methods.

In Germany, a new “protection against infection act” (IfSG) became operative in 2001 including reporting of norovirus infections to the health authorities. Between 2001 and 2010, a considerably increasing number of norovirus infections have been reported (2001: 4178; 2010: 145.221) to the Robert Koch Institute. Norovirus is now the most commonly reported cause of infectious diseases in Germany. Using molecular amplification techniques, sequencing and phylogenetic analysis on samples of norovirus outbreaks during the last ten winter seasons the fluctuating circulation of genogroup I and II strains and the emergence of new drift variants and recombinant strains have been analysed by the Consultant Laboratory for Noroviruses in Germany. Altogether, about 80 to 95 % of the outbreaks were caused by genogroup II strains with the predominance of several drift variants of GII.4 during high epidemic norovirus seasons. Genotype II.7/II.6 and several other recombinant genogroup II strains were less common but were circulating more frequently in low epidemic seasons. This presentation will review the epidemiological data on the burden of norovirus infections in the German population as well as data on strain typing and molecular epidemiology over the past decade.

O1.2 – Prevalence and Genetic Diversity of Noroviruses in Healthy School Children in Cameroon

James Ayukekbong[1], Magnus Lindh[1], Nancy P. Nenonen[1], Ferdinand Tah[2], Theresa Nkuo-Akenji[3] & Tomas Bergström[1]

[1]University of Gothenburg, Gothenburg, Sweden, [2]Camyaids Institute of Laboratory Diagnosis and Clinical Research, Douala, Cameroon, [3]Department of Microbiology, University of Buea, Cameroon

Norovirus is considered as the main cause of non-bacterial gastroenteritis (GE) worldwide, but the natural course of this infection is incompletely known. Previously, we have reported that one third of healthy school children in Cameroon carried norovirus at a single occasion. Here, we have studied the seasonality of norovirus carriership in the tropics. Preliminary results at two different time points in a community in Cameroon show a drop of norovirus prevalence from 29% to 5% in samples collected in October, 2009 and in September, 2011 respectively. The most prevalent strains were homologous to clinically dominating norovirus strains associated with GE worldwide. This result may suggest a cyclic prevalence of norovirus in healthy children in Central Africa. In contrast, prevalence of enteroviruses remained unchanged at 32%. A prospective longitudinal study is ongoing to clarify this seasonality influence of norovirus spread in the tropics.

Reference:
Ayukekbong J, Lindh M, Nenonen N, Tah F, Nkuo-Akenji T, Bergstrom T: Enteric viruses in healthy children in cameroon: Viral load and genotyping of norovirus strains. *J Med Virol* 2011, 83(12):2135-2142.

O1.3 – Comparison of Two Norovirus Outbreaks in the Gothenburg Region

Nancy P. Nenonen, Charles Hannoun & Tomas Bergström

Dept of Infectious Diseases/Virology, University of Gothenburg, Sweden

Two widespread community outbreaks of norovirus gastroenteritis are described showing the differing characteristics and properties of NoV GI and GII strains in environmental settings. Diverse GI NoV strains were detected in patient faeces sampled during a non-seasonal community outbreak of waterborne gastroenteritis affecting approximately 2400 individuals in Lilla Edet in the Gothenburg region. Genotyping based on sequencing and cloning of major capsid regions confirmed a great diversity of NoV GI strains noted in initial patient samples, where GI.3, I.4, I.7, and a new genotype, proposed GI.9 were detected. Detection of multiple NoV GI strains in the Lilla Edet outbreak contrasts sharply with the findings from a widespread seasonal outbreak of NoV GII.b infections where contaminated

confectionary products affected approximately 300 individuals in Frölunda, Gothenburg. The point source of the NoV GII infections proved to be the master baker. These two outbreaks exemplify the contrasting epidemiology of the GI and GII norovirus infections.

O1.4 – Development of a Next Generation Sequencing Pipeline for Norovirus from Clinical Samples

Julianne R Lockwood[1], Matt Cotten[2], Ian Goodfellow[3], Paul Kellam[2,4] & Judith Breuer[4]

[1]Virology Department, Great Ormond Street Hospital for Children, London, UK, [2]Pathogen Genetics, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, UK, [3]Section of Virology, Department of Medicine, Imperial College London, London, UK, [4]Division of Infection and Immunity, University College London, London, UK

Noroviruses represent one of the most common diseases of mankind yet our understanding of their molecular evolution and the viral factors that contribute to the widespread dominance of the GII.4 isolates is currently lacking. Here we report the development of a next generation sequencing (NGS) pipeline to allow the rapid generation of norovirus full genome sequences from clinical samples. We aim to use this approach to generate large datasets that facilitate a wider understanding of intra-host transmission dynamics, sequence bottlenecks, viral recombination and, importantly, if common viral sequences contribute to the high prevalence of GII.4 noroviruses.

To our knowledge this is the first report of NGS for norovirus full genome sequencing. We have sequenced full genomes from 13 stool samples. We generated cDNA using random hexamers and amplified the full genome by PCR using norovirus-specific primers to generate overlapping amplicons. The amplicons were pooled, purified and sequenced using the Roche 454 next generation sequencing platform. The stool samples were collected from five immunosuppressed patients from three wards during an outbreak at Great Ormond Street Hospital over a two month period in 2010. We have obtained full genome coverage from all 13 samples: single isolates for 2 patients and longitudinal isolates (two to six) for three patients, collected over 4-8 weeks.

We are currently comparing *de novo* and reference based algorithms for assembling the genomes and for documenting the diversity within each patient's virus population.

We are using the sequencing data generated to elucidate several important features of norovirus infection. We would like to understand the phylogenetic relationship of norovirus between patients and to viruses circulating in the community, especially for the design of measures to stop these infections in health care facilities. These data will also be important for defining factors that control sequence variation of norovirus within patients.

Our long-term aim is to develop a next generation sequencing pipeline that facilitates norovirus full genome sequencing in a cost-effective and clinically relevant time frame.

O1.5 – P2 Domain Profiles and Shedding Dynamics in Prospectively Monitored Norovirus Outbreaks

Faizel H.A. Sukhrie[1,2], Peter Teunis[3,4], Harry Vennema[1], Jolanda Bogerman[5], Matthias F.C. Beersma[2] & Marion Koopmans[1,2]

[1]Laboratory for Infectious Diseases and Perinatal Screening, Centre for Infectious Disease Control (RIVM), Bilthoven, The Netherlands, [2]Department of Virology, Erasmus Medical Center, Rotterdam, The Netherlands, [3]Epidemiology and Surveillance Unit, Centre for Infectious Disease Control (RIVM) Bilthoven, The Netherlands, [4]Hubert Department of Global Health, Rollins School of Public Health, Emory University Atlanta, GA, USA, [5]Municipal Health Service, Rotterdam, The Netherlands

Background: Norovirus P2 domain sequencing is commonly used to extrapolate transmission events within an outbreak setting. The current definition is that transmission among cases is considered to be proven when no sequence variation is found. Previous studies have shown a high mutation rate and errors during replication of the norovirus genome, therefore the validity of this cut-off must be evaluated.

Methods: P2 domain sequences were obtained from persons sampled during 3 prospectively monitored outbreaks within the region of Rotterdam (Netherlands). Upon detection of two or more PCR confirmed cases in the same ward, an enhanced outbreak investigation protocol was applied. Fecal samples were collected from all consenting patients and healthcare workers who had potentially been exposed and all samples were tested by RT-PCR for presence of norovirus RNA. Norovirus positive persons were followed up weekly, until they tested negative for norovirus. Each strain was typed using polymerase gene based typing, and sequencing of the P2 domain to identify variation within and between patients and outbreaks. Thereby sequence data could be linked to estimated total virus excretion in individual subjects.

Results: In all the studied GII.4 outbreaks including HCW and patients, P2 domain variation was found. Virus excretion and sequence variation showed a mixed pattern.

Conclusion: P2 domain variation is common during outbreaks involving HCW and patients, and increases with duration of virus shedding. Therefore, the currently used cut-off should be relaxed to accommodate minor sequence variation in identifying clusters of norovirus infection. When using sequence data to support outbreak investigations, sequence diversity should be interpreted in relation to target gene and timing of sampling since onset of illness.

O1.6 – Descriptive Analysis of a Cohort of Chronic NoV Patients (n=40) in a Tertiary Care Hospital Setting (2003-2011)

Mathias F.C. Beersma[1], Faizel H.A. Sukhrie[1,2], Jean Luc Murk[1] & Marion Koopmans[1,2]

[1]Department of Virology, Erasmus Medical Center, Rotterdam, The Netherlands, [2]Laboratory for Infectious Diseases and Perinatal Screening, Centre for Infectious Disease Control (RIVM), Bilthoven, The Netherlands

Background: Norovirus (NoV) has emerged as a major cause of non-bacterial gastro-enteritis (GE) in the community and in health care settings. Although awareness for outbreaks and sporadic cases of NoV has significantly increased, the occurrence and disease burden of chronic NoV infections in immuno-compromised patients is underappreciated.
Methods: We studied chronic NoV infections in a tertiary care setting during 2003 and 2011. Prolonged (chronic) NoV infection was defined by a positive PCR for NoV with cycle threshold (CT) <=25 one month after onset of disease.
Results: During 2003-2011, 732 patients with NoV were diagnosed. A total of 40 patients (5.5%) developed chronic NoV infection. All patients with chronic NoV were immuno-compromised; 19 patients had hematological diseases, 15 patients underwent solid organ transplantation, and 6 patients had other diseases. Clinical symptoms ranged from mild abdominal complaints to severe diarrhea with nausea, cachexia and dehydration. Eight patients (20%) excreted NoV more than 6 months; 5 of them more than 1 year. The mean CT value in serial stool samples of individual chronic patients ranged between CT 13 and CT 27 (mean CT 21.9). Very low CT values (CT <17) occurred during both the acute (<3 months) and chronic (>= 3 months) stages. Mortality within 3 months after the last positive stool sample was relatively high for the hematological patients when compared to other patients (46% (9/19) versus 4.8% (1/21), OR 18, p=0.01 (CI: 1.0 to 324).
Conclusion: The results show that chronic NoV is a frequent and potential life-threatening infection among severely immuno-compromised patients. Identification of risk factors and development of therapeutic interventions are urgently needed.

O1.7 – A New Method for the Evaluation of Hand Disinfectants against Norovirus Simulating Practical Conditions: The Modified EN 1500 Test

Maren Eggers[1], Katrin Steinhauer[2], Martin Enders[1] & Miranda Suchomel[3]

[1]Laboratory Prof. G. Enders & Partner, Stuttgart, Germany, [2]Schülke & Mayr GmbH, Norderstedt, Germany, [3]Institute for Hygiene and Applied Immunology of the Medical University of Vienna, Austria

Norovirus outbreaks can cause widespread and intractable outbreaks among patients and medical staff in the winter months. Direct transfer from one person to another is one of the most significant ways to become infected with norovirus. Thus, hand hygiene could be an important method for prevention and control of norovirus gastroenteritis outbreaks in healthcare settings. However, non-enveloped viruses such as the norovirus are particularly resistant against disinfectants and to prevent and control the spread of nosocomial norovirus infections, disinfectants with proven virucidal efficacy must be used. Therefore, disinfectants must pass a virucidal activity test performed in compliance with good laboratory practise and country-specific standards. For instance, the European Committee for Standardization Technical Committee 216 – Chemical Disinfectants and Antiseptics (CEN/TC 216) developed an European standard that comprises a virucidal quantitative suspension test for chemical disinfectants and antiseptics used in human medicine (prEN 14476:2011). But a standardized virucidal hand disinfection test on the whole hand simulating practical conditions as the European standard EN 1500 for determining the bactericidal effectiveness of hand rub agents is still lacking.

The aim of this study was to establish a virucidal hand disinfection test according to EN 1500. Twenty human volunteers had their hands contaminated with the murine norovirus strain S99 Berlin since the human norovirus cannot grow in cell culture. We assessed the norovirus effectiveness of two commonly used hand rubs (80 g ethanol and or 45 g 2-propanol und 30 g ethanol) and compared these results with the EN 1500 reference alcohol [2-propanol, 60% (v/v)]. The reduction factor of both products should be superior or the same as the reference alcohol for acceptance. Both hand rubs passed the test. However, ethanol has greater activity than n-propanol against non-enveloped viruses whereas n-propanol has slightly greater activity against bacteria. Subsequently, we repeated the tests with ethanol [70% (w/w)] as reference and only the ethanol based hand rub has been shown to significantly reduce the titer of norovirus recovered from contaminated hands. In conclusion, we have successfully established a virucidal practical hand test according to EN 1500 but our findings have to be confirmed in European ring trials.

Session 2 – Immunology and Vaccine Development

O2.1 – Cruising with Noroviruses: Mechanisms of Immune Evasion

Ralph S. Baric

Department of Epidemiology, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Noroviruses are the major cause of epidemic viral gastroenteritis, worldwide. Outbreaks occur in communities, families, recreational facilities, retirement communities, military settings, day care centers, hospitals and schools. These small structured RNA viruses are estimated to cause ~200,000 deaths/year, primarily in young infants and immunosenescent populations, who are most at risk for severe disease outcomes, especially in the developing world. Human noroviruses are very heterogeneous in nature consisting of two major genogroups that differ by ~50% or more in the sequence of the major capsid protein, VP1. Each genogroup is further subdivided into numerous genoclusters which differ by 20% or more in the major capsid protein. Noroviruses bind to histoblood group antigens (HBGA), likely as host cell receptors or co-receptors that mediate virus docking and entry into cells. It is likely that variant patterns of norovirus-HBGA interaction influence virus distribution and outbreak patterns in natural populations. We illustrate that the major epidemic strain GII.4, which causes ~80% of the cases of epidemic disease, are evolving by epochal evolution and undergoing antigenic variation in the face of human herd immunity. Using panels of time-ordered mouse and human monoclonal antibodies and virus like particles (VLPs), we identify major evolving epitopes associated with HBGA blockade immune responses. Using chimeric norovirus VLPs, we demonstrate that the major evolving blockade epitopes are portable and can be moved across time-ordered VLP strains. Using this information, we describe and compare the efficacy of monovalent and multivalent vaccines in mouse immune models, providing a platform approach to rapid vaccine design. Our data support the hypothesis that norovirus vaccines can be designed with significantly improved homologous and heterologous cross blockade immune responses and which may significantly reduce the considerable disease burden associated with these important human pathogens.

O2.2 – Challenges and Solutions on Vaccine Development against Noroviruses

Xi Jiang

Division of Infectious Diseases, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH, USA

Noroviruses (NoVs), an important cause of epidemics of non-bacterial acute gastroenteritis, remain difficult to study due to the lack of an efficient cell culture and animal model for NoVs. Despite these difficulties great progresses have been made in development of a non-replicable subunit vaccine against NoVs, including the production of virus-like particles (VLPs) by variable eukaryotic expression systems and the clinical trials of the VLP vaccine against the prototype Norwalk virus. However, due to the wide spread natures and the high disease burden of NoV gastroenteritis, new vaccines with higher cost-effectiveness are highly demanded. In addition, NoVs are genetically diverse and vaccines against major circulating types, particularly the predominant GII.4 NoVs, need to be included. This presentation summarizes our research progresses on 1) development of a new candidate subunit vaccine, the P particle of NoVs, and 2) establishment of a human volunteer challenge model for a GII.4 virus. The NoV P particle is formed by 24 copies of the protruding (P) domains of the NoV capsid protein VP1. It is easily produced, stable, and highly immunogenic, which are being evaluated as the second generation vaccine for NoVs. In addition, the P particles are a useful vaccine platform to present foreign antigens. Two examples of chimeric vaccines containing the rotavirus spike protein VP8* and the influenza virus M2e proteins respectively will be discussed. The human volunteer challenge study of the GII.4 virus (a 2003 isolate) was performed among 40 healthy adults between two groups (23 secretor vs.17 non-secretor). Of the 23 secretors, 14 (61%) shed virus, 13 (57%) became ill (vomiting and/or diarrhea) and 56% had 4 fold increase in GII.4 antibody. In contrast, only one of the 17 non-secretors became ill, one other shed virus for a single day ($P<0.001$ for each variable), but none had seroconversion. Saliva of all the 23 secretors but none of the 17 non-secretors bound the P particles of the challenge strain. 74% of the secretors but none of the non-secretors had pre-existing anti-GII.4 antibodies by the receptor-blocking ELISA. This challenge model would be useful for future evaluation of vaccines against NoVs.

O2.3 – The Role of Type I Interferon in Persistent Murine Norovirus Infection

Alexander J. Chettle, Amita Shortland*, Barbara Blacklaws*, Anne Cooke#, Clare Bryant* & Jonathan L. Heeney**

**Department of Veterinary Medicine, University of Cambridge, Cambridge, UK,*
#Department of Pathology, University of Cambridge, Cambridge, UK

Persistent viruses have evolved many mechanisms to evade the host immune response including alteration of the host's ability to produce or respond to type I interferon. Certain strains of murine norovirus (MNV) are able to persist for months following infection, whereas other strains are cleared within days. However, the reasons for these differences and how the host immune system may be involved are not well understood. The importance of type I interferon in the immune response to MNV infection has been demonstrated by findings that type I (along with type II) interferons inhibit the translation of MNV proteins during infection[1] and that the virus encodes a protein whose functions include delaying the upregulation of IFN-beta following infection.[2] We have recently characterised a persistent strain of MNV, known as O7, and have compared the effects of infection with this strain against infection by the acute strain MNV-1.CW3. We have demonstrated that both immortalised cell lines and primary bone marrow-derived macrophages initiate a significantly reduced type I interferon response on infection by O7 compared with MNV-1. Furthermore, a comparison of the immune response of bone marrow-derived macrophages with bone marrow-derived dendritic cells revealed different response patterns to the virus strains. Our results indicate an enhanced ability of persistent strains of MNV to inhibit induction of type I interferon across certain cell types, which may contribute to the establishment of persistent infection.

References:
[1]Changotra, H et al. (2009) "Type I and type II interferons inhibit the translation of murine norovirus proteins." *Journal of Virology* 83: 5683–5692
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O2.4 – Influence of Vaccination Strategies on IgG Fc Glycosylation

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It has now been established that antibodies play an important role in the protection against virus infections, e.g. HIV (1, 2). Antibodies can neutralize the interaction of the virus with the receptor on target cells but may also be involved in the elimination of viruses. IgG antibody responses are also investigated after vaccination against Norovirus (3, 4).
However, the pathogenic potential of IgG antibodies is highly dependent on the IgG subclass and on the IgG Fc glycosylation (5-7). IgG Fc de-fucosylation highly increases the effector function of IgG antibodies to recruit killer cells in tumor therapy and IgG Fc de-galactosylation and de-sialylation increases the inflammatory potential of IgG antibodies as described e.g. for arthritis patients. We have investigated IgG Fc glycosylation after different T cell dependent and independent vaccination protocols and found great differences in the development of inflammatory or anti-inflammatory IgG antibodies, which might have an important impact also on the development of vaccination protocols against Norovirus.

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Session 3 – Virus-Receptor Interactions

O3.1 – Conservation and Flexibility of the HBGA-Binding Interfaces of Noroviruses: Two Essential Strategies of Norovirus Survival in Human Hosts

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Noroviruses (NoVs), the major cause of viral acute gastroenteritis in humans, recognize histo-blood group antigens (HBGAs) as receptors or attachment factors through their surface protruding (P) domains. Crystallography of the NoV P domain-HBGA complexes indicated that the HBGA binding interface of a NoV is composed of multiple amino acids that form a complex interaction network with the terminal saccharide residues of individual HBGAs. While some of these amino acids are evolutionary highly conserved, other residues are highly variable. To elucidate the biological significance of these two features, we performed extensive mutagenesis analyses on individual amino acid residues in and around the HBGA-binding interface for their roles in interaction with HBGAs. Four NoVs in both genogroup (G) I (GI.1 Norwalk virus) and GII (GII.4 VA387, GII.9 VA207 and GII.10 Vietnam026) were studied. Our data demonstrated that single mutations at the amino acids constituting the core structure of the HBGA-binding interface completely wiped out the binding function of the mutants and these amino acids are evolutionary highly conserved. On the other hand, mutations at amino acids around the core structure changed the HBGA binding pattern of the mutants and/or altered the HBGA binding affinity of the original binding pattern. These amino acids are highly variable, which may confer NoVs an enormous flexibility. These data suggest that both features are important for the persistence of NoVs in human populations. While the conservation of the HBGA-binding interfaces is essential for NoVs to survive, the flexibility enables the single-stranded RNA NoVs to adapt to new hosts with additional HBGA variations. Such adaptation could be driven by an alternative selection pressure, such as the herd immunity from the human hosts, which will be discussed.

O3.2 – Studies of Norovirus Interactions with Host Glycoconjugates Using Virus-like Particles: What Is Happening over Time?

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Norovirus is the main cause of viral gastroenteritis worldwide and appears both as spontaneous outbreaks from contaminated food and water supplies as well as pandemics appearing with increased intensity every other year and with the highest incidence during the winter season in Europe. Although most healthy citizens stand the acute infection through substitutions with water and salts without sequelae, children, elderly, cardiovascular diseased and immunosuppressed patients, especially those out of reach for healthcare or at institutions, are at high risk for developing complications, chronic infections and even a fatal outcome. Today there is no efficient anti-viral treatment available but large efforts are motivated to find efficient vaccines or drug prophylaxis for controlling disease spreading.

While lacking efficient cell-culture models for studies of human norovirus propagation *in vitro*, we have focussed on studying the binding characteristics of various virus-like particles (VLPs) to define attachment factors among glycoconjugates presenting histo-blood group antigens (HBGA) e.g. saliva from single individuals, neo-glycoproteins and glycosphingolipids (GSLs) from natural sources. GSLs offer unique possibilities for structural and functional studies since they have, per molecule, only one oligosaccharide chain sticking out of the membrane and a lipophilic ceramide part that anchors the GSL into the membrane. The attachment and passage through the host cell membrane is a critical step in the virus infectious cycle and studies of virus to membrane interactions offer special challenges and advantages due to the unique presentation of the oligosaccharide ligands. Examples of different experimental methodologies e.g. ELISA[1], Chromatogram Binding Assay (CBA)[2], QCM-D (Quartz Crystal Microbalance with Dissipation) [3] and TIRFM (Total Internal Reflection Fluorescence Microscopy)[4] as well as of computer simulations[4] of norovirus capsid protein P2 domains interactions with carbohydrate ligands will be presented. Special focus will be given to time-resolved processes of virus-ligand interactions.

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O3.3 – Insights into Norovirus-Carbohydrate Interactions at Atomic Resolution from NMR

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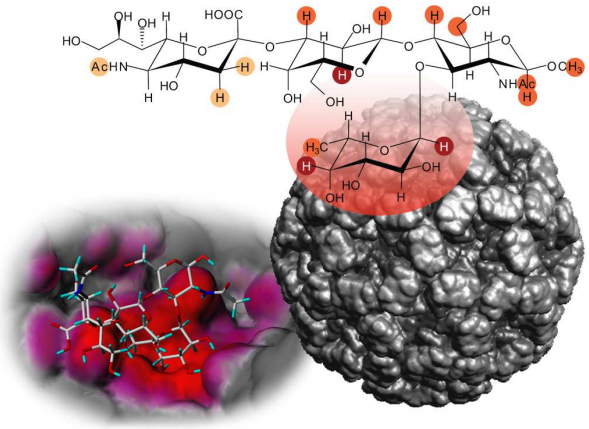
Many viral infections are initiated by attachment of viruses to host cells via carbohydrate-protein interactions. Noroviruses are a prominent example where the virus in a first step attaches to histo-blood group antigens located on the host-cell surface. One therapeutic option to prevent norovirus infection is to inhibit this first step of infection by administering drugs that block the carbohydrate-binding pocket on the surface of the norovirus. In order to develop such entry inhibitors knowledge about the characteristics of the protein-carbohydrate recognition process is required. It will be demonstrated that ligand-based NMR experiments such as STD NMR deliver important information on virus-host-cell recognition at atomic resolution. With STD NMR it is possible to identify those portions of the glycan chains on a host cell that make contact with invading noroviruses, and transferred NOE experiments deliver information about bioactive conformations of the glycan attachment factor. Importantly, these experiments can be done with native viruses or virus like particles without the need to prepare individual hull proteins. Using Noroviruses as an example it is demonstrated how a comprehensive picture of the interaction of these viruses with the human glycome can be obtained. In addition, the information generated is a starting point for the development of novel entry inhibitors. The cartoon below shows sialyl Lewis^x interacting with a Norovirus particle.



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O3.4 – Murine Noroviruses Bind Glycolipid and Glycoprotein Attachment Receptors in a Strain-Dependent Manner

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Human noroviruses use various carbohydrates, including histoblood-group antigens as attachment receptors. Due to a lack of an efficient cell culture system or small animal model for human noroviruses, little is known about the biological role of glycan binding during infection. Murine noroviruses (MNV) also use carbohydrates during attachment, particularly terminal sialic acids on gangliosides, present on murine macrophages. Murine noroviruses (MNV) are also enteric viruses that bind to cell surface glycans but in contrast to their human counterparts can be grown in tissue culture and a small animal host. In this study, we determined glycan-binding specificities of the MNV strains MNV-1 and CR3 *in vitro*, identified molecular determinants of glycan binding, and analyzed infection *in vivo*. We showed that unlike MNV-1, CR3 binding to murine macrophages was resistant to neuraminidase treatment and glycosphingolipid depletion. Both strains depended on N-linked glycoproteins for binding, while only MNV-1 used O-linked glycoproteins during attachment to macrophages. *In vivo*, CR3 showed differences in tissue tropism compared to MNV-1 by being able to replicate in the large intestine. Mapping of a glycan-binding site in the MNV-1 capsid by reverse genetics identified a region topologically similar to the HBGA-binding sites of the human norovirus strain VA387. The recombinant virus showed distinct changes in tissue tropism compared to wild-type virus. Taken together, our data demonstrates that MNV strains evolved multiple strategies to bind different glycan receptors on the surface of murine macrophages, and that glycan binding contributes to tissue tropism *in vivo*.

O3.5 – Recognition of Cell-Membrane Mimics Containing Glycosphingolipids by Norovirus GII.4 Virus-like Particles

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Artificial model membranes produced by self-assembly of amphiphilic molecules provide an excellent platform for studying the interaction between virions and cell membranes. These cell-membrane mimics make it possible to present a ligand of interest in a more native-like environment while preserving potentially relevant characteristics such as membrane fluidity, ligand mobility or the ligand's ability to cluster into microdomains.

In this work, model membranes, in the form of supported phospholipid bilayers or phospolipid vesicles containing small amounts of glycosphingolipids (GSLs), are used to investigate carbohydrate recognition by virus-like particles (VLPs) from the GII.4 norovirus strain.

In a first example, we identify by thin-layer chromatography, galctosylceramide (GalCer) as a ligand for the VLPs. Real-time binding studies using a quartz crystal microbalance combined with atomic force and fluorescence microscopy of GalCer-containing supported lipid bilayers further reveal that a clustered arrangement of the glycosphingolipids plays a crucial role in promoting a firm attachment of the pathogen to the lipidic membrane, most likely via the establishment of multiple contacts between the particle and the membrane. Moreover, the VLPs attach preferentially to the rim of gel-phase domains, indicating that firm binding is only possible under optimal ligand arrangement in the membrane, highlighting the crucial role of ligand presentation, organization and mobility in fine tuning the strength of a multivalent interaction.

We further investigate the interaction between individual fluorescent GSL-vesicles and surface immobilized VLPs with single virus particle sensitivity. Our newly developed assay, based on total internal reflection fluorescence microscopy, allows for the quantification of affinities and binding energies between GSL-containing membranes and the VLPs. Kinetics analysis over large time scales yields information on multivalency, on the presence of domains and the role of cell-membrane curvature.

As exemplified here, fluidic artificial membranes have a unique potential in providing fundamental understanding on the importance of the membrane's physico-chemical properties in tuning host recognition by a viral pathogen.

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Session 4 – Food Safety

O4.1 – A Two-Year Systematic Study to Assess Norovirus Contamination in UK Oysters

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Contamination of bivalve shellfish with norovirus from human faecal sources is recognised as a major human health risk. Risk assessment and management currently relies on the use of *Escherichia coli*. However, this approach has been repeatedly demonstrated to inadequately contain the risk from human enteric viruses. Considerable progress has been made towards development of detection methods for norovirus in molluscan shellfish and the European Committee on Normalisation has a working group addressing the development of a standard method, which is due for publication in 2012. EU texts foreshadow the adoption of virus controls when the methods are sufficiently developed and viral controls may soon be adopted into Community legislation. It has therefore become essential to gain information about the application of the methods and potential impact of legislative standards on shellfisheries.

This two year study aimed to address this issue by subjecting monthly samples of oysters from 39 production areas around the UK, selected to ensure a representative selection of likely faecal contamination levels, to testing for norovirus genogroups I and II using a quantitative real-time RT-PCR method. Norovirus was detected in 76.2% (643/844) of samples but detection exhibited a marked seasonality with a positivity rate of 90.0% for samples taken between October and March. Quantification of positive samples revealed that the majority were below the limit of quantification (100 genome copies/g digestive tissues) for both genogroups. However a number of samples contained levels in excess of 10,000 copies/g. As with prevalence, average quantities varied markedly between seasons, with highest levels detected between December and March. All sites tested provided at least one positive result; prevalence varied from 21% to 100%. Levels varied markedly between sites with some sites scoring consistently over 1,000 copies/g during the winter while others rarely or never exceeded 100 copies/g.

This study also examined the relationships between norovirus and potential risk indicators. Correlations were found between norovirus and both harvesting area classifications and *E. coli* when data was analysed by site rather than by sample. Strong correlations between norovirus contamination and environmental temperatures were also found. On this basis we were able to elaborate a predictive method for determining sampling risk scores based on a combination of risk factors. This approach could assist risk management in relation to, for example, highlighting the need for virus monitoring or enhanced operator control measures.

In summary this study, the largest of its type undertaken to date, provides a systematic analysis of norovirus contamination in classified oyster production areas in the UK. The data will help risk managers in the UK and elsewhere to formulate strategies to reduce the risk of human illness resulting from viral contamination of bivalve molluscs.

O4.2 – Identification of Noroviruses in Food – Finnish Experiences

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Virus detection from foodstuff is fastidious, since enrichment by cell-culture is not presently possible. In our studies, attempts to detect noroviruses from food samples linked to gastroenteritis outbreaks started in 1997. Viruses were eluted from food (mostly raspberries and imported oysters) with glycine-buffer and concentrated into low volumes using different methods. In recent years, PEG-precipitation for soft fruit and proteinase K -treatment for oysters were applied. Nucleic acid was extracted with magnetic silica before one-step real-time RT-PCR. In 2009-2010, noroviruses were found 9/100 (9%) food, mostly berry samples; with GI in 7 and GII in 2 samples. Before 2009, norovirus detection in berries succeeded only twice. In all cases, the norovirus genogroups in patients and food matched. In oysters, noroviruses were detected in 4/7 batches in 2010. Before 2010, they were found in oysters linked to 5 outbreaks. Efforts to increase sensitivity of viral analyses are still needed.

O4.3 – Persistence of Human Norovirus GII.4 and GI.4, Murine Norovirus, and Human Adenovirus on Soft Berries as Compared with PBS at Commonly Applied Storage Conditions

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An estimated 20 % of hNoV outbreaks in Europe are foodborne. Fresh produce, especially raspberries, is a common vehicle for the transmission of human norovirus (hNoV), whereas strawberries, even though consumed more frequently and more prone to contamination, have not been associated with hNoV outbreaks to date. The attribution of hNoV illness to food is, however, challenging, due to e.g. difficulties in detecting hNoV, the absence of a systematic surveillance for foodborne viral disease, and the fact that secondary person to

person transmission may mask foodborne transmission of hNoV. As a consequence, the contribution of food matrices in causing foodborne hNoV outbreaks is not clear and the significance of foodborne hNoV disease to public health may be estimated incorrectly. Quantitative microbial risk assessment (QMRA) can be used to aid the estimation of the infection risk of foodborne hNoV disease and to elucidate the role of different food commodities in the transmission of hNoV. QMRA, however, requires vast amounts of data, such as data on the persistence of viruses in the food chain.

HNoV persistence on fresh raspberries post-harvest is challenged by the applied storage conditions such as temperature and relative humidity, the duration of storage (shelf life), and the fruit matrix itself. Therefore the persistence of hNoV GII and GI, murine norovirus (MNV-1, a culturable surrogate of hNoV), and human adenovirus (hAdV, an indicator for human fecal contamination), on raspberries, strawberries and in phosphate buffered saline (PBS) at 4 °C, 10 °C and 21 °C, mimicking commonly applied storage conditions was studied by molecular and cell culture techniques. Monophasic, biphasic and Weibull models were fitted to virus counts with maximum likelihood estimation.

The tested viruses demonstrated the highest persistence in PBS followed by raspberries and then strawberries. D-values (the time required for the first 1 log10-unit reduction in virus titer) of all viruses exceeded or reached the shelf life of berries, however, at room temperature a sharp decrease in infectious MNV-1 and hAdV particles on strawberries was observed with D-values of only 1 day, and 2 days for hNoV GI based on the targeted genome fraction. The greater viral persistence on raspberries compared to strawberries at room temperature may at least in part explain the differences in the number of associated outbreaks. Our results show in addition that already low contamination levels of the highly infectious hNoV may be associated with an infection risk of humans after consumption of soft berries, especially raspberries. The estimated viral decay rates and uncertainties serve as important input requirements in the quantitative assessment of public health risks from consumption of soft fruits.

O4.4 – Detection and Quantification of Norovirus in Raspberries Implicated in Disease Outbreaks

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In recent years, raspberries have emerged as high risk foods for norovirus (NoV) outbreaks. However, the lack of robust and standardized methods has limited our understanding of the level of NoV contamination of raspberries correlated to public health risk. We present an optimized and quality controlled protocol for the viral RNA extraction using NucliSens and Plant RNA Isolation Aid and detection by RT-qPCR including bovine serum albumin of NoV in 25 g of raspberries. The protocol was applied on raspberries that had been linked epidemiologically to nine NoV outbreaks which had occurred in Denmark from 2009-2011. To minimize false negative results, mengovirus and murine norovirus were evaluated as sample process control viruses (SPCVs). Using the median RT-qPCR recovery for either SPCV±2 SD as quality criteria for successful extraction, 98% of samples were approved. The 50% limit of detection was 119 and 140 RT-qPCR units/25g for NoV GI or GII, respectively. Nineteen (37%) of 51 raspberry samples tested positive for GI and/or GII NoVs with the respective geometric mean values of 70 (range 22-330) or 29 (range 3-217) detectable genome copies/g of raspberries, when corrected using mengovirus recoveries. A 100% identical GI.6 sequence was detected in both raspberries and patient stool samples associated to one outbreak. In conclusion, this is the first report demonstrating quantified virus levels of contaminated raspberries linked to illness. These data can contribute to the development of quantitative risk assessments and improve our understanding of public health risk related to different levels of NoV contamination in raspberries.

Session 5 – Structural Biology and Antiviral Drug Discovery

O5.1 – Structural Biology of Caliciviruses

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Caliciviruses consisting of five major genera constitute a family of positive-sense single-stranded RNA viruses that infect a wide variety of hosts including humans. These viruses are non-enveloped, icosahedral viruses typically 380-400 Å in diameter. Viruses in the *Norovirus* (NoV) and *Sapovirus* genera, are mostly human pathogens, whereas viruses in the other three genera, *Lagovirus*, *Vesivirus and Nebovirus*, are animal pathogens. Noroviruses exhibit considerable genetic diversity and based on phylogenetic analyses, the *Norovirus* genus is classified into two genogroups (GI and GII) that contain mostly human strains, and three additional groups that include bovine (GIII), feline/canine (GIV), and murine (GV) strains. Caliciviruses exhibit two distinct types of genome organization. In the *Norovirus* and *Vesivirus* genera, the genome is organized into three open reading frames (ORFs), whereas in the other three genera, the genome is organized into two ORFs. In all cases, however, the calicivirus RNA encodes a large polyprotein, the major capsid protein VP1 (55 to 70 kD), and a basic minor structural protein VP2. In the *Norovirus* and the *Vesivirus* genera, the large polyprotein, VP1 and VP2 are encoded separately by ORF1, ORF2 and ORF3, respectively. In contrast, in the *Sapovirus* and *Lagovirus* genera, the polyprotein and the major capsid protein VP1 are contiguously encoded by ORF1, and VP2 is encoded by ORF2. In all caliciviruses, the polyprotein is post-translationally processed by the viral protease, which itself is a component of the polyprotein, into several non-structural proteins (NSPs) including a polymerase and 2C-like p41. In recent years, there has been a significant progress in our understanding of the structural biology of caliciviruses in general and noroviruses (NoV) in particular. In addition to atomic level description of various calicivirus capsids, structural studies have provided insight into the structural basis of capsid-related functions such as strain-dependent interactions between NoV and histo-blood group antigens (HBGAs), the interplay between antigenicity and HBGA recognition in the epochal evolution of GII.4 NoV, and also functional aspects of some of the NSPs that are critical for viral replication. This talk will review the recent advances in the calicivirus structural biology and point to future directions needed to further a complete structural understanding of the many functions critical for NoV replication and pathogenesis.

We acknowledge support from NIH grant (P01AI057788) and R. Welch Foundation (Q1279).

O5.2 – Structure-Based Inhibitors of Norovirus RNA-Dependent RNA-Polymerases

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Caliciviridae are RNA viruses with a single-stranded positively-oriented polyadenylated genome, responsible for a broad spectrum of diseases such as acute gastroenteritis in humans. Recently, analyses on the structures and functionalities of the RNA-dependent RNA-polymerase (RdRp) from several Caliciviruses have been reported. The RdRp is predicted to play a key role in genome replication, as well as in synthesis and amplification of additional subgenomic RNA.
Starting from the crystal structures of human norovirus RdRp (hNV), we performed an in silico docking search to identify synthetic compounds with predicted high affinity for the enzyme active site. The best ranked candidates were tested in vitro on murine norovirus (MNV) and hNV RdRp to assay their inhibition of RNA polymerization. The results of such combined computational and experimental screening approach led to the identification of two high-potency inhibitors: Suramin and NF023, both symmetric divalent molecules hosting two naphthalene-trisulfonic acid heads. We report here the crystal structure of MNV RdRp alone, and in the presence of the two identified inhibitors. Both inhibitory molecules occupy the same RdRp site, between the fingers and thumb domains, with one inhibitor head close to residue 42 and to the protein active site. To further validate the structural results, we mutated Trp42 to Ala in MNV RdRp, and the corresponding residue (i.e. Tyr42 to Ala) in hNV RdRp, showing decrease of inhibitory potency of the two compounds for both the mutated proteins.

O5.3 – RNA-Protein Interaction Networks Required for Norovirus Replication: Towards Development of Antivirals

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Murine norovirus (MNV), is currently used as model for elucidating molecular details of human noroviruses (HuNV) replication due to its similarity and the fact that MNV can be propagated in cell culture, has reverse genetics systems and an available animal model (Vashist et al., 2009). The 5'- and 3'- extremities of MNV genome have been shown to be conserved and important for viral replication (Bailey et al., 2010; Simmonds et al., 2008). Using RNA-affinity column chromatography and mass spectrometery, we identified various

host proteins that interact specifically with the 5'- and the 3'- extremities of MNV genome and confirmed the interaction during infection using Co-IP RT-PCR. *In silico* analysis showed that most of these proteins interact with each other revealing an interaction network between 5'- and 3- end. Using siRNA knock down a few of these proteins including La, PTB, DDX3, hsp90 etc. were shown to be required for the MNV replication. Small chemical molecules binding to DDX3 and hsp90 were shown to affect viral replication. Further work on Hsp90 inhibitor indicated that it is required for viral encapsidation. This small molecule has potential to be used as effective antiviral against HuNV.

Session 6 – Molecular Biology of Norovirus

O6.1 – Norovirus Chimaeras: Are They Useful for Studying Gene Function?

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Noroviruses are a diverse group of positive-strand RNA viruses that share a very similar genome structure and organisation and thus belong to the same genus within the *Caliciviridae*. Noroviruses are further divided into 5 genogroups (I to V) on the basis of phylogeny, each with distinctive biological properties and tropisms. RNA recombination is a major driving force in virus evolution and phylogenetic studies have established that naturally occurring chimeras or recombinants occur between noroviruses although these do appear to be limited to specific groupings of the viruses. The absence of a cell culture system for the enteric noroviruses has meant that research into the biological activity of proteins encoded by the genes of these viruses has been restricted. The discovery of murine norovirus (MNV) and its subsequent adaptation to growth in cell culture (Wobus *et al.*, 2004) was a major scientific advance and MNV is now a useful model for studying basic aspects of norovirus replication.

Recombination in naturally occurring enteric noroviruses occurs at hot spots in the genome and the recombinants are likely generated through a copy- choice mechanism where the RNA-dependent RNA-polymerase jumps between different templates during genome replication. In noroviruses the recombination hot spot is located at or near the ORF1-ORF2 junction (Bull *et al.*, 2007) and this appears to be mediated by the presence of RNA loop structure(s) (Simmonds *et al.*, 2008). A novel recombinant has recently been created by forcing two variants of MNV to infect the same host cell and the recombination point was located to the ORF1 -2 junction (Mathijs *et al.*, 2010). The ability to construct mutants and to 'cross' genetic variants is potentially a powerful tool for studying gene function but it requires specifically defined biological properties and a selection system. In viruses with small compact genomes, viral protein precursors are often multifunctional and have subtle

and complex interactions with the host cell, therefore using systems reliant on recovery of viable progeny (following recombination) to define gene- function is likely to be very limiting. Reverse genetics offer an alternative and more precise way to generate recombinants and such systems are available for MNV (Ward *et al.*, 2007;Chaudhry *et al.*, 2007) allowing the possibility to make recombinant genomes to study the biological properties of viral proteins.

We are interested in developing inhibitors for the human norovirus protease and recently described the atomic structure of the 3C protease from the genogroup I human norovirus, Southampton Virus (SV) (Hussey *et al.*, 2010a;Hussey *et al.*, 2010b). As part of a study to investigate the factors determining the specificity of this protease we have started to generate chimeras of the MNV genome with the SV protease gene. We noted that SV protease cleaves the MNV N-terminal protein from the 2C protein via the AEGP recognition site. Bioinformatic analysis of the MNV 1 genome identified a further potential AEGP cleavage site within the N-terminal protein but this does not appear to be cleaved by the SV protease. Therefore we used our reverse genetic system to knock out the site within MNV and were still able to recover viable MNV. Taken together these data show that site specificity for the protease is determined by multiple factors and the presence of a perfect cleavage site is not a predictor that the site is a substrate for the protease.

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O6.2 – Using Proteomics, Reverse Genetics and Animal Models to Understand Noroviruses

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The inability of human noroviruses to be efficiently propagated in cell culture has, until relatively recently, delayed studies on the molecular mechanisms of norovirus translation, replication and pathogenesis. The discovery of murine norovirus (MNV) has resulted in significant advances in the study of the viral and host factors that play important roles in the norovirus life cycle.

We have recently developed a number of efficient and robust reverse genetics systems[1,4,6] for MNV that have enabled us to begin to dissect the role of viral sequences in the norovirus life cycle and viral virulence. Using proteomics and reverse genetics we have identified a number of host factors important for the norovirus life cycle and further demonstrated that modifications to RNA structures can lead to alterations in viral virulence.[2] We have previously shown that the norovirus VPg protein is essential for viral protein synthesis and interacts with the host cell cap-binding protein eIF4E but that this interaction is not essential for viral translation, at least *in vitro.*[3] Our recent studies have indicated that VPg also interacts with the eIF4G protein directly and that siRNA mediated reduction in eIF4GI or eIF4GII has an inhibitory effect on viral translation and replication. Mutational analysis has also been used to identify residues within VPg that contribute to virus translation via an interaction with eIF4G but also those that contribute to viral replication and VPg nucleotidylation. We have also generated a number of experimental systems including replicons and epitope tagged infectious noroviruses that are currently being used to further dissect the life cycle of these important pathogens.

We have recently identified the first innate immune regulator for any member of the *Caliciviridae* family. This novel protein, referred to as VF1, is found within the subgenomic RNA of murine norovirus and contributes to viral virulence in an immunocompromised mouse model.[5] By developing a reverse genetics system for a persistent norovirus, namely MNV-3 we have also now further shown that VF1 contributes to the establishment of viral persistence in an immunocompetent genetic background. Proteomics has been used to identify a number of interacting host factors and to begin to understand the mechanism of action of this novel innate immune regulator.

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O6.3 – Differences in AUG Recognition for the Termination Reinitiation Mechanism of Different Caliciviruses

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Calicivirus structure proteins are expressed from a subgenomic mRNA with two overlapping cistrons. The first open reading frame of this RNA codes for the viral major capsid protein VP1 and the second for the minor capsid protein VP2. Translation of VP2 is mediated by a termination/reinitiation mechanism, which depends on an upstream sequence element of about 40 to 80 nucleotides denoted “termination upstream ribosomal binding site” (TURBS). Three short sequence motifs within the TURBS were found to be essential for reinitiation. Motif 1 is conserved among caliciviruses and is complementary to a region within helix 26 of mammalian 18S rRNA. It tethers the post-termination ribosome to the RNA to give time for reloading of translation initiation factors. Motif 2 is located about 12 to 23 nucleotides upstream of the VP2 start codon and the complementary motif 2* can be found directly upstream of motif 1. The latter two motifs hybridize and thereby form a secondary structure that probably positions post-termination ribosomes in an optimal distance to the VP2 start codon.

We analyzed the termination-reinitiation mechanism of VP2 translation for human Norovirus (huNV). In contrast to other caliciviral genera the stop codon of the VP1 coding frame is located upstream of the VP2 start codon, which points at differences in approaching the start codon. Therefore, we have focused on the reinitiation event and found differences in AUG recognition between the different caliciviruses but also common requirements for this interesting translation initiation mechanism.

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O6.4 – Unified Proposal for Norovirus Genotyping and Nomenclature

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Since the mid 1990s, norovirus genotypes were genetically classified on the basis of their complete capsid gene (ORF2) sequences. New genotypes were assigned when sequences were found to differ by >20%[1] at the amino acid (aa) level from their nearest neighbour. With the rapid accumulation of sequence data around the world, the genotype cut-off threshold was adjusted to a minimum of 15% pairwise difference.[2] However, this threshold was arbitrary, since noroviruses were shown to evolve over time. Moreover, in our analyses, using a representative genotype strain, which is defined by "the oldest sequence available for that genotype", the distance cut-offs did not hold for strains from several genotypes. In addition, the lack of an internationally-accepted standard for noroviruse (NoV) nomenclature and cluster definition has led to conflicting genotype nomenclature in the literature.[2, 3]

The need for common classification standards was recognized at the 3rd and 4th International Conference on Caliciviruses in Mexico (2007) and Chile (2010). An international norovirus working group was assembled to develop standards for a universal nomenclature and typing system. Comparative analysis was performed on datasets containing all currently recognized NoV strains using distance plots and phylogenetic analysis. Based on these analyses, it was decided that phylogenetic clustering, combined with relative distance, would be the standard for defining a new genotype.

Starting from 2007 (Fields reference table[4]), a new genotype will be assigned if at least two geographically diverse complete capsid sequences are detected. The clustering should be robust using more than one accepted phylogenetic method, including a branch support test. Using the inferred distances from these phylogenetic methods, the average distance between sequences of the new Genotype cluster and sequences of the nearest genotype cluster cluster, should not overlap within two SD of each other. Proposed nomenclature and reference strains resulting from discussions within the working group will be publicly available and regularly updated on the Noronet typing tool website.[5]

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Session 7 – Pathogen-Host Interactions in Crohn's Disease

O7.1 – Intersection of Murine Norovirus with the Atg5-Atg12/Atg16L1 Autophagy Protein Complex

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Human noroviruses are the major cause of epidemic non-bacterial gastroenteritis worldwide and are an important cause of sporadic gastroenteritis. However, little is known about the mechanisms of host resistance to human noroviruses, in part due to the lack of an efficient tissue culture system and a small animal model. The related murine norovirus (MNV) can be cultured in myeloid cells and infects mice. The replication of MNV in macrophages is associated with extensive membrane rearrangements that generate membranous replication complexes (Hyde et al., 2009; Sosnovtsev et al., 2006; Wobus et al., 2004). Some of the membranes induced during MNV replication are reminiscent of the double membrane-bound organelles called autophagosomes.

Autophagy and autophagy proteins play important roles in host defense against infection, development, cellular energy homeostasis, and multiple diseases including cancer and inflammatory bowel disease. The degradative function of autophagy involves delivery of cytoplasmic cargo contained within autophagosomes to the lysosome. This process requires the ordered activity of protein complexes that induce autophagosome formation, envelopment of specific cargo or bulk cytoplasm, elongation and closure of autophagosome membranes, fusion of the outer autophagosomal membrane to the lysosome, and degradation of cargo within the autophagosome (Virgin and Levine, 2009; Fujita et al., 2008b). One protein complex required for this degradative autophagy pathway contains Atg16L1 bound to a covalent Atg5-Atg12 conjugate that is generated by the action of Atg7.

Using MNV, we have recently defined a novel, non-degradative role for the Atg5-Atg12/Atg16L1 autophagosome elongation complex in type II interferon (IFNγ)-mediated host defense. The direct antiviral activity of IFNγ against MNV in macrophages requires Atg5-Atg12, Atg7, and Atg16L1, but did not require induction of autophagy, the degradative activity of lysosomal proteases or fusion of autophagosomes and lysosomes. Furthermore, when compensatory type I interferon responses are absent, Atg5 expression in macrophages and/or granulocytes is essential for restricting MNV replication *in vivo*, as well as preventing lethal MNV infection. IFNγ, via the Atg5-Atg12/Atg16L1 protein complex, inhibits the formation of the cytoplasmic MNV replication complex in macrophages, likely accounting for the Atg5-dependent effects we observed on multiple subsequent steps in the MNV life cycle

including synthesis of negative-sense RNA, synthesis of positive-sense RNAs and viral structural proteins, and viral replication. Interestingly, using stochastic optical reconstruction microscopy we observed co-localization of Atg16L1 and the MNV polymerase, suggesting that Atg5-Atg12/Atg16L1 protein complex, which is required for the blockade of MNV replication by IFNγ, may be found at the MNV replication complex. Thus, proteins required for the highly-conserved and essential autophagy pathway likely have unique roles in host defense against noroviruses.

O7.2 – Host-Microbiome-Virome Interactions in the Intestinal Tract: IBD and Beyond

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Recent advances have enabled a comprehensive understanding of the genetic architecture of inflammatory bowel disease (IBD) with over 30 identified and replicated disease loci. , The pathophysiological consequences of disease gene variants in Crohn disease and ulcerative colitis, the two main subentities of IBD, so far are only understood on the single disease gene level, yet complex network analyses linking the individual risk factors into a molecular risk map are still missing. Many of the identified genes are involved in basic biological processes that evolved either at the unicellular or early multicellular level, e.g. cytoskeletal dynamics, autophagy, altered structural integrity of epithelial cells, the related secretion and composition of extracellular matrix components and primordial (innate) immune responses. Many of these cellular programs govern the interaction between the host and the environment and are pivotal for survival. The host is in continuous contact to a microbiota and virus-laden environment and clear evidence for a host-genetic control of the resident microflora has been presented already in basal metazoans. Vice versa, the physiological co-evolved microbial communities on epithelial interfaces are important for cellular fate decisions and, ultimately, for the fitness of the host. However, the precise mechanisms of the genome-genome interplay between host and pathogenic or commensal microbiota and the consequences for the local immune response at epithelial barriers are much more complex than described in the current simple models, yet it seems evident that a defective holobiome (i.e. a dysbalanced host-microbiota “symbiosis”) causally contributes to disease manifestation and progression. Interestingly it seems that the resident microbiota and possibly also viral communities do not only represent an extended individual phenotype of the host, but indeed contain information that may transmit the phenotype if transplanted into another individual.

O7.3 – Colonic Mucosa-Associated Microbiota Is Influenced by an Interaction of Crohn's Disease and FUT2 (Secretor) Genotype

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The FUT2 (Secretor) gene is responsible for the presence of ABO histo-blood group antigens on the gastrointestinal mucosa and in bodily secretions. Individuals lacking a functional copy of FUT2 are known as "nonsecretors" and display an array of differences in susceptibility to infection and disease, including Crohn disease. To determine whether variation in resident microbial communities with respect to FUT2 genotype is a potential factor contributing to susceptibility, we performed 454-based community profiling of the intestinal microbiota in a panel of healthy subjects and Crohn disease patients and determined their genotype for the primary nonsecretor allele in Caucasian populations, W143X (G428A). Consistent with previous studies, we observe significant deviations in the microbial communities of individuals with Crohn disease. Furthermore, the FUT2 genotype explains substantial differences in community composition, diversity, and structure, and we identified several bacterial species displaying disease-by-genotype associations. These findings indicate that alterations in resident microbial communities may in part explain the variety of host susceptibilities surrounding nonsecretor status and that FUT2 is an important genetic factor influencing host–microbial diversity.

Session 8 – Other Caliciviruses

O8.1 – *Cis*-Acting RNA Structures Promoting Translation Reinitiation in Caliciviruses

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Caliciviruses use a termination/reinitiation mechanism for expression of their minor capsid protein VP2. This process is dependent on defined sequence motifs in a region located upstream of the VP2 translational start site which is called the 'termination upstream ribosomal binding site' (TURBS). This element is necessary to tether the posttermination ribosome to the viral RNA to allow reloading of initiation factors and restart of translation. In general, the molecular principles of this mechanism show similarity to prokaryotic translation initiation. The talk will summarize basic features of this interesting mechanism of gene expression and present the results of RNA structure and function relationship of the TURBS.

O8.2 – Infection of Calves with Bovine Norovirus GIII.1 Strain Jena Virus: An Experimental Model to Study the Pathogenesis of Norovirus Infection

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Course of disease, clinical signs and viral fecal shedding were examined following experimental infection of newborn calves with bovine norovirus (NV) GIII.1 strain Jena (JV) to evaluate if this infection could be a useful experimental model for human NV. For this study, twenty two clinically healthy, newborn calves were used; ten for passaging virus for the inocula and twelve for the experimental infections. Fecal samples collected from a calf (calf 1481) during the acute phase of diarrhea contained the most viruses. Samples were pooled for the preparation of a standard inoculum.

The reverse transcription-PCR (RT-PCR) and sequencing applied to amplicons derived from these samples confirmed the presence of bovine NV GIII genotype 1. By TEM, only NV particles were present and no bacteria were detected by routine microbiological screening.

Calves were 2.5 to 3 hours (h) old when they arrived at the animal facility. They were each kept in confinement using individual pens. Serum samples were collected upon arrival to evaluate the immunoglobulin status and the presence of bovine NV-specific antibodies. Calves were treated once with enrofloxacine to minimize secondary bacterial infections. Six calves were infected with inoculum containing JV and 6 calves were mock infected with PBS only (control calves). Two hours later, calves were fed 2 liters of colostrum followed by normal cow's milk, twice daily. Two calves infected with JV were euthanized and necropsied at: 12 hpi (before the onset of diarrhea), 18 to 21 hpi (within the diarrheic period) and at 4 days pi (dpi) when diarrhea had abated. Pairs of control calves were processed at the same time as infected animals.

Calves infected with JV developed severe watery diarrhea between 14 and 16 hpi and this symptom lasted for 53.5 to 67.0 hours. Fecal material and intestinal contents were examined for JV by RT-PCR and ELISA assays. Fecal shedding of JV started between 12 and 16 hpi. Of the calves observed to 4 dpi, one stopped shedding at 23 hpi and one continued shedding until necropsy at 4 dpi. Throughout the trial none of the control calves tested positive for JV by ELISA or RT-PCR. Antigen ELISAs for common endemic pathogens revealed no bovine coronavirus, group A rotavirus, *E. coli* F5 or cryptosporidia in the faecal swabs from any calf.

In conclusion, this homologous large animal model resembles in many aspects the disease observed after NV infection of humans and may therefore be a useful experimental model for human NV infection.

For literature, see the accompanying abstract by Liebler-Tenorio et al. below.

O8.3 – Infection of Calves with Bovine Norovirus GIII.1 Strain Jena Virus: Intestinal Lesions and Distribution of Viral Antigen

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The interaction of norovirus with the intestinal barrier was investigated in a homologous infection model in calves. One group of six newborn calves were inoculated orally with bovine norovirus GIII.1 strain Jena virus (JV) and another group of six calves served as mock-inoculated controls. Following infection, calves were euthanized and necropsied before onset of diarrhea (12 hours post inoculation, hpi), shortly after onset of diarrhea (18-21 hpi) and post-convalescence (4 days pi, dpi). At necropsy, samples were collected from the duodenum, mid and distal jejunum, Peyer's patches in jejunum and ileum and mesenteric lymph nodes. Macroscopically, increased liquid and foamy ingesta were seen in the small intestine and extended into the colon at 18-21 hpi. Histological lesions were limited to the small intestine. Mild villus atrophy was seen as early as 12 hpi in one of the two inoculated calves. Severe villus atrophy was present in both inoculated calves at 18-21 hpi. The stunted villi were covered by irregular, attenuated epithelium and had frequently denuded tips. At 4 dpi, the intestinal mucosa had recovered to normal morphology. Viral antigen (JV antigen) was detected in paraffin sections by the indirect immunoperoxidase method using a primary antibody against the capsid antigen of JV. It was seen in the cytoplasm of epithelial cells on villi at 12 hpi and 18-21 hpi. In addition, granular material positive for JV antigen was detected in macrophages in the lamina propria of villi from 18 hpi to 4 dpi. There was no indication of systemic infection as described for norovirus infection in mice. In the mock inoculated controls, no clinical signs, intestinal lesions, or viral antigen were observed in the tissue sections.

The findings confirm that bovine norovirus of newborn calves can be used to study the pathogenesis of norovirus infection, to determine target cells for viral replication and to study the development of immunity.

Reference:

Otto PH, Clarke IN, Lambden PR, Salim O, Reetz J, Liebler-Tenorio EM: Infection of calves with bovine norovirus GIII.1 strain Jena Virus: an experimental model to study the pathogenesis of norovirus infection. J Virol 85/22: 12013–12021, 2011

Session 9 – Calicivirus Evolution and Epidemiology

O9.1 – Potential Involvement of Histo-Blood Group Antigens (HBGAs) in Host-Calicivirus Co-Evolution

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Many caliciviruses of the norovirus and lagovirus genus (RHDV) bind to HBGAs, which show a polymorphism within the host species. The strains of norovirus that bind to HBGAs can collectively infect all humans but each strain infects a subgroup of the population only, suggesting a past co-evolution of humans and noroviruses that led to a trade-off where the human population is partly protected whilst the virus circulation is maintained. We termed "Herd Innate Protection" the partial protection provided by the HBGAs polymorphism. Given its recent emergence, its high virulence and its ability to bind to HBGAs, RHDV is expected to exert a strong selective pressure on some glycosyltransferase genes of rabbits, providing a model suitable for studying calicivirus-host co-evolution based on field observations. Our recent results using this host-pathogen pair provide evidence for evolution of the virus ability to recognize the host HBGA diversity and for strain-dependent selection at the a1,2-fucosyltransferases locus and of ABO phenotypes following outbreaks. This suggests that a host-pathogen co-evolution involving HBGA recognition is indeed taking place.

O9.2 – Lessons from Integrated Molecular and Epidemiological Surveillance of Noroviruses in an International Context

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Noroviruses have been recognized in the past decade as important causes of infectious intestinal disease, affecting persons of all age groups, but with particular health impacts in young children, elderly and persons with immunocompromised states. Through a collaborative network of epidemiologists and laboratory scientists we have collected data on reported outbreaks since 1999 to get a basic understanding of diversity of noroviruses and how this affects their epidemiology and impact. Multiple genogroups and genotypes have been described, but genotype II.4 noroviruses have been dominant since the emergence of a particularly transmissible variant in 2002. GII4 noroviruses evolve through accumulation of mutations that affect binding and antigenicity of the viruses, and are common causes of outbreaks in healthcare institutions. Greater diversity is seen in reported food and waterborne outbreaks, including the identification of complex recombinant viruses reflecting simultaneous exposure of humans with virus mixtures. This may occur in diffuse foodborne

outbreaks, where contamination occurred through sewage exposed water, or for instance in groups of children which have the highest infection incidence in the population. Understanding these fundamental aspects of norovirus molecular epidemiology will help target control and prevention measures.

O9.3 – Intraindividual Noroviral Evolution Analyzed by Next Generation Sequencing

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Norovirus has become an important cause for infectious gastroenteritis. Chronic infections have been described recently and will become more prevalent with increasing numbers of immunocompromized patients. Here, we studied noroviral evolution of 3 GII.4 strains during chronic infection. The great advantage of deep sequencing is its ability to recognize even low prevalence quasispecies in a sample. Thus also the variability within a sample is examined.
We tested 5, 4, and 4 samples from three chronically infected transplant patients. The specimens covered a period of 252 to 456 days. Amplicons comprising 760 nt and labeled with barcodes on both ends served as templates for sequencing. A total of 34,000 reads were analyzed from a mixture of 13 PCR products. Consensus sequences of all samples were used for genotyping and multiple sequence alignment, including phylogenetic analysis.
About 80% of the reads could be uniquely aligned to our reference strain Norovirus Hu/GII-4/Aichi1/2008/JP. All consensus sequences could be categorized as Genotype II.4 strain, 2006b variant. The evolutionary rate increased over time for all 3 individuals on amino acid level. Individual 2 accumulated a higher proportion of nonsynonymous mutations than patient 1 and 3, also reflected by a tendency to positive selection within this individual. Most longitudinal changes in the epitope ligation score are decreases, indicating escape from T cell selective pressure.
To our knowledge this is the first study applying next generation sequencing to analyze the evolution of noroviral strains in several chronically infected patients with > 3 longitudinal samples. We hypothesize that the rising evolutionary rate of nonsynonymous mutations in all three individuals results from selective pressure exerted by the host´s immune response that leads to the ultimate clearance of the infection.

O9.4 – Molecular Evolution of GII-4 NoV Strains

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Background: We have previously shown through analysis of the hypervariable P2 region of the capsid protein (VP1), that a reservoir of randomly generated genetic diversity is maintained in the circulating norovirus population. We predicted that antigenically novel GII-4 strains emerge from this pool of genetic variants, through a selective process mediated by host immunological pressure. Our data suggests that this selection is focused on two surface-exposed loops (Site A and Site B) in the P2 domain, each one three amino acids in length, which function as GII-4 variant-specific epitopes.

Methods: In this study, we aimed to further characterise the diversity at these epitope sites among circulating GII-4 norovirus strains. To do this, we developed a sequencing-by-synthesis method, specifically targeting the two nine-nucleotide regions encoding the Site A and Site B epitopes, thus removing the need to sequence long regions of the capsid gene (ORF2). This allows high throughput testing of large numbers of strains rapidly and more economically. We then used this method to screen a panel of 958 GII-4 norovirus isolates from clinical specimens collected 2001-2011.

Results: Sequence analysis revealed 59 different Site A/Site B amino acid motif combinations. We found that the Site A could be grouped into 3 clusters (1, 2, and 3) since the year 2000 to date based on surface area motifs, and the viruses within each were predicted to be antigenically similar. Overall, Site B showed greater diversity than Site A, and no clear clustering into particular surface motifs.

Conclusions: Transition from one surface motif to another at site A correlated with worldwide epidemics, and possibly correlated with significant changes at a major epitope, whereas increased diversity at Site B was associated with localised epidemics. Also, diminished diversity within Site B was associated with low or baseline epidemic cycles. This correlated with the different role of the two putative antigenic sites which had been confirmed in serological assays.

POSTER ABSTRACTS

P01 – Prolonged Norovirus Shedding in Renal-Transplanted Patients

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Background: Noroviruses are common cause of gastroenteritis in both children and adults. Although the illness is generally mild and of short duration, new evidence suggests that the illness can be severe, especially among young children, the elderly and immunocompromised persons. In patients after kidney transplantation a chronic norovirus infection with prolonged virus shedding could develop.

Methods: To detect norovirus RNA in stool samples, real-time RT-PCR protocol, published by Kageyama et al. (2003) was performed. In the period of two and a half years, from June 2009 through December 2011, the stool samples, from 13 renal transplant recipients with chronic norovirus excretion were analysed.

Results: Observed chronically infected patients were shedding norovirus from 1 to 18 months (mean 7.7 months) with relatively constant dynamic of norovirus excretion at concentration of 10^6-10^7 genome copies/ml. The genotype GII.1, GII.4, GII.7 and GII.17 were found, respectively. Comparison of capsid sequence alignments of a 343 nucleotide fragment from earliest to last sample available from each patient, demonstrated only minor differences in nucleotide sequences. All patients remained infected by the initially detected strain throughout the observing period. In two patients out of 13 with genotype GII.4 infection, an evidence for nosocomial transmission was determined.

Conclusion: Norovirus infection may persist in renal-transplanted persons with or without clinical symptoms for months. These patients may serve as reservoirs for nosocomial norovirus infection and require contact isolation. Healthcare personnel should be aware of the possible risk in virus transmission and should inform patients about appropriate hygiene measures. Preventive strategies for norovirus transmissions and hospital hygiene procedures should be applied as such chronic infections can affect the transplantation outcome.

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[2]Schorn R, Höhne M, Meerbach A et al. Chronic norovirus infection after kidney transplantation: molecular evidence for immune-driven viral evolution. Clinical Infectious Diseases 2010; 51: 307-314.

P02 – Risk of Norovirus Genotype II.4 Infection Increases with Age

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Background: Norovirus (NoV) is one of the major causes of diarrhoea throughout the world, both in community settings and in hospital outbreaks. Although several NoV genotypes exist, genogroup II genotype 4 (GGII.4) is the predominant cause of hospital outbreaks of NoV gastroenteritis. The reason for this is currently not known.

Objectives: To describe the distribution of NoV genotypes in Danish hospital and community settings. Secondly, to examine if any association can be identified between the odds of infection with NoV GGII.4 and the age and setting of the patient, e.g. hospital or general practitioners (GP).

Study design and analysis: Stool samples received for gastroenteritis diagnostics from 2006 through 2010 and found positive for NoV, were eligible for the study. If several samples were tested, only the first NoV positive stool sample from each patient was included. Genotyping analysis was successfully performed on 1040 of 2533 hospital samples and 905 of 1143 stool samples obtained from GP. Hospital samples were mainly chosen for genotyping analysis based on their geographical distribution and ward within each hospital. Statistical analyses were performed using logistic regression on a multilevel model.

Results: NoV GGII.4 was found in 92% of the hospital samples and 56% of samples from GP. A significantly higher proportion of older patients (>60 years of age) contracted GGII.4 compared with the infant group (0 – 2 years), both from hospitals *(OR: 18.1, p-value: <0.001, 95% CI: 7.7 – 42.7)* and GP *(OR: 5.7, p-value: <0.001, 95% CI: 3.1 – 10.6)*. In our analysis, an effect of setting alone on the odds of infection with NoV GGII.4 could not be established *(OR: 0.98, p-value: 0.95, 95% CI: 0.6 – 1.8)*.

Conclusions: This study confirms that most NoV infections in hospitals are caused by GGII.4 whereas in the community, half of NoV infections are caused by other genotypes. Furthermore, the study gives strong evidence that the risk of infection with NoV GII.4 increases with age. The reason for this is unknown. It could be caused by a more efficient transmission, host cell invasion, or an increased immune evasion capability of the NoV GGII.4 variants. Further research into this is needed.

P03 – Detection and Genetic Diversity of Human Calicivirus Circulating in Germany

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Human noroviruses (NoV) have emerged as the most common cause of gastroenteritis in all age groups and mass outbreaks pose an increasing health problem even in Germany. Sapoviruses (SaV), primarily associated mainly with sporadic gastroenteritis in young children, are increasingly reported as causative agent of gastroenteritis outbreaks in children and adults. In Germany, NoV infections of all age groups were notified nationwide by the German Disease Prevention Act (Infektionsschutzgesetz, IfSG) whereas no specific surveillance has been conducted so far to study the circulation of SaV.

In our lab, circulating NoV and SaV were analyzed by qRT-PCR, RT-nested PCR, and direct sequencing for genotyping in the capsid and the polymerase gene region. For genotyping of NoV, a total of 3140 NoV positive stool specimens from 843 outbreaks collected between July 2001 and June 2011 were analyzed. Comparison of the phylogenetic data of the previous 10 NoV winter seasons revealed a high diversity and considerable changes of the genotype/subtype distribution. Genotype II.4 variants predominated in 7 out of 10 seasons (> 60%) attended by remarkably high NoV activity in Germany. In contrast, a higher diversity of genotypes was observed in winter seasons with moderate reporting of norovirus infections (2003/2004; 2005/2006, and 2010/11).

For the detection of SaV, a retrospective study of 570 NoV-negative stool specimens of gastroenteritis outbreaks and sporadic cases collected between January 2005 und December 2010 was performed. Overall, 47 SaV positive samples from 24 outbreaks and 5 sporadic cases were detected and sequenced. 66 % of the patients were age 1 -10, 4% were age 11-20, and 23 % were between 21 and 97 years old. The analysis showed that SaV circulating in Germany exhibit a high genomic diversity (genotypes I.1, I.2, I.5, II.1, II.2, and V) and is causative also for gastroenteritis outbreaks in children and adults.

P04 – Role of Norovirus in Acute Gastroenteritis Development in the Northwest of Spain during 2010-2011 Period

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Nowadays, NoV are recognized as common cause of sporadic gastroenteritis in all age groups worldwide (Parashard *et al.,* 2007; Hall *et al.,* 2011). However, the real prevalence of NoV as a cause of sporadic cases of gastroenteritis has been poorly documented and is probably under-appreciated in most regions of the world. In this study, we analysed samples from patients affected from gastroenteritis who seek medical care in A Coruña, Galicia (Northwest of Spain), to determine relative role of NoV as a causal agent of acute gastroenteritis in this region.

A total of 2690 specimens were routinely tested for bacterial pathogens. When it was required Rotavirus (RV) and Adenovirus (AdV) were tested by EIA. All these specimens were also analysed for NoV genogroups I and II (GI and GII) detection by real-time RT-PCR (rtRT-PCR) with TaqMan probe. NoV were detected in 747 (27.77%) of these samples. The etiologic agent causing the illness was determined in 39.77% of the total gastroenteritis cases analysed.

This is the first study carried out in Galicia, and to our knowledge in Spain, that determine the role of NoV in illness development. Our results show that development of sensitive clinical assays for NoV identification and a widespread use of these assays will help to close the gap on sporadic cases of acute gastroenteritis and guide more appropriate case management.

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P05 – Surveillance of Noroviruses in the Czech Republic, 2008 to 2011

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Background: Noroviruses are the most common cause of gastroenteritis outbreaks in industrialised countries. Gastroenteritis caused by a norovirus infection has been described as a highly seasonal syndrom and a disease of adolescents.
Methods: The Public Health Service has systematically collected reports of norovirus gastroenteritis since 2008 throughout the Czech Republic. The Medical Faculty Department of Epidemiology has systematically collected reports of norovirus diagnostics in individual Czech laboratories since 2008; the Department is diagnosing these infections at the Clinic of Infectious Diseases in Pilsen, too. We analysed all the data.
Results: There were reported 857-1801 cases of norovirus gastroenteritis in individual years (morbidity 8.2-17.1/100,000 inhabitants). Eleven cases - 0.2% of the total - were lethal. Among all reported cases there were 61.2% associated with outbreaks; the number of reported outbreaks has increased from 17 to 22 per year. An average of 39.0% of all cases were 65 years or older. Among children under 5 years noroviruses were the sixth frequent cause of gastroenteritis. In all years these infections were more frequent (73.8%) in colder months (October-March). The number of laboratories diagnosing norovirus infections has increased from 8 to 37, they used mostly immunochromatograpic methods and ELISA tests. Among hospitalized children, noroviruses were the second most frequent etiological agent of gastroenteritis (5.6-15.5%) at the Clinic of Infectious Diseases.
Conclusion: Similar to other European countries, noroviruses are an important cause of gastroenteritis in the winter and among older people. The increase of reports could be associated with the higher numbers of laboratory confirmed cases and better quality of reports.

P06 – High Prevalence of Norovirus and Low Prevalence of Rotavirus in Hospital and Community Wastewater after Introduction of Rotavirus Vaccine in Nicaragua

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Norovirus (NoV) and Rotavirus (RV) are major causes of pediatric diarrhea and are altogether associated with approximately 800,000 deaths in young children every year. In Nicaragua, previous studies have shown a high prevalence of both NoV and RV in children with severe diarrhea. In October 2006, a national RV vaccination program using the pentavalent RV5 vaccine from Merck was implemented. Clinical studies have shown a reduction of RV associated diarrhea, but the burden of diarrhea in the country remains at similar levels as compared to the pre-vaccination years.

In this study, we investigated the role of NoV and RV in Nicaragua post RV vaccination. We did this by detecting and quantifying NoV and RV in hospital and community wastewater in the city of León from July 2007 to July 2008 using ultracentrifugation and real-time PCR techniques. The detected strains were further characterized by nucleotide sequencing. Measuring enteric viral levels in wastewater samples is an indirect way to monitor the presence of these enteric agents in the local population.

The major finding was the high prevalence of NoV in comparison to RV in all sampling points (44% vs 11%, $p<0.05$). In hospital wastewater (n=28), NoV was detected in 46% of the samples whereas RV was detected in 7%, while in incoming wastewater (n=28), NoV was detected in 54% as compared to 23% for RV. Furthermore, the concentration of NoV was generally higher as compared to RV. NoV strains were detected throughout the year, of which a majority (20/21) of the characterized strains were of genotype GII.4, with one strain belonging to genotype GII.3. In contrast, RV was observed mainly during the rainy season (July–September), and the only partially characterized sample was similar to a bovine gene of the vaccine strain (98% nt identity).

We conclude that the transmission of NoV is high in the community in Nicaragua after RV vaccination. Although clinical cases of RV diarrhea have reduced, the burden of diarrhea in the country remains high. The results of this study highlight the need for continuous assessment of NoV following RV vaccine introduction.

P07 – Evaluation of a Transcription-Reverse Transcription Concerted Assay (TRCRtest NV-W) for Norovirus Detection in Stools

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Standardized application format, automated sample preparation and rapid detection of norovirus (NoV) in stools are essential to provide an early and accurate diagnostic service in both sporadic gastroenteritis and outbreak prevention and management. The aim of this study was to evaluate the performance of a new diagnostic assay (TRCRtest NV-W, Tosoh Corporation, Tokyo, Japan) that uses the transcription-reverse transcription concerted (TRC) method for isothermal amplification and real-time detection of NoV in stools.

The specificity of the TRCRtest NV-W was confirmed by the absence of positive signals for archived stools containing enteric viruses other than NoVs, while it was able to detect all the different NoV GI and GII genotypes tested. Clinical evaluation of TRCRtest NV-W performed in comparison with a conventional RT-nPCR on RNAs extracted by NucliSens easyMAG system (BioMérieux) from 377 of 387 fresh stools (10 turned out inhibited by RT-nPCR were excluded from the evaluation), belonging to subjects with gastroenteritis, showed no significant difference in detection rate between the two detection methods (13.8% *vs* 14.6%: $P = 0.83$). At the retesting of the 21 (5.6%) discordant samples (9 false positive and 12 false negative), clinical evaluation showed that there were still 1 false positive and 12 false negative.

As the false positive specimen was resolved in favour of TRCRtest NV-W by the analysis of the amplicon and 5 out of the 12 false negative specimens gave positive results on RNA extracted by a manual method (QIAamp Viral RNA Mini Kit, Qiagen), the concordance, sensitivity, specificity, positive and negative predictive values of the TRCRtest NV-W increased from 94.4%, 78.2%, 97.2%, 82.7% and 96.3%, respectively, at the original testing, to 98.1%, 89.1%, 100%, 100%, and 97.8%, respectively, after discordant analysis. These results together with the ease to use and rapid turnaround time (within 1 h) of TRCRtest NV-W assay allow it to be proposed as a useful tool for the detection of NoV from stools in clinical virology laboratories.

P08 – The Development of LENTICULES™ as Reference Materials for Noroviruses

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International guidance on microbiological testing and laboratory accreditation requires that quality systems include provision for method validation/verification and internal and external quality control. For this it is further recommended that, where possible, traceable reference materials (RMs) are utilised. LENTICULES™ are control-dried plano-convex discs containing biological material in a solid water soluble matrix (Codd *et al* 1998). They were initially developed by the U.K. Health Protection Agency (HPA) as an alternative method for the preservation of bacteria to assist in standardisation of quantitative methods in food microbiology. The aim of this study was to examine the potential for LENTICULES™ to be used to stabilise noroviruses (NoV) for use as candidate RMs in quantitative real-time RT-PCR assays.

Noroviruses used in this study originated from human faecal material, screened for the absence of other faecally transmitted pathogens. The norovirus strains present in the faecal material were characterised by sequencing and samples containing GI and GII strains representative of genotypes commonly circulating in the community were selected. RMs were produced utilising modified lenticulating technology. A batch comprising 500 LENTICULES™ containing both norovirus genogroups was produced according to ISO Guide 34 (Anonymous, 2009). The batch was tested and quantified using an ISO 17025 accredited quantitative real-time RT-PCR assay. Sufficient homogeneity was established using procedures described by Fearn and Thompson (2010), while stability at <-15ºC and ambient temperature (17-22°C) was assessed over 52 weeks and 7 days respectively.

Lenticulation was shown to be an effective means of preservation of detectable noroviruses. LENTICULES™ were sufficiently homogeneous and stable throughout medium term frozen and short term storage at room temperature to serve as RMs. Virus LENTICULES™ have the advantages of being easy to manipulate, provide assigned values and do not require the manipulation of high titre clinical material by the testing laboratory.

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P09 – A Comparative Analysis of Noroviruses and Human Adenoviruses Dissemination: From Raw Sewage to River Water

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Fecal contamination of water and food may allow the transmission of human viral pathogens such as human adenoviruses (HAdV) and noroviruses (NoV). In order to improve water and food safety, human adenoviruses (HAdV) were previously proposed as viral indicators of contamination (Pina et al. 1998). Here, we present a comparative surveillance of NoV and HAdV dissemination in sewage treatment plants (STP) and river water receiving STP effluents.

In an initial study sewage samples were collected in two different treatment plants at the entry and after tertiary treatments based on UV disinfection (STP1) and a combination of UV and sand filtration (STP2). NoVGGI and NoVGGII were detected by nPCR in 50 and 66.6% respectively of entry samples in STP1 and 80 and 100% in STP2. Their presence was also detected in reclaimed water from both treatment plants showing 50 and 80% of positive results respectively in STP2. Mean values of NoVGGII were quantified in selected reclaimed water samples by qPCR as $3.55x10^6$ GC/L. HAdV were positive by qPCR in all tested sewage samples showing reductions between 1.5-2 log from entry (mean value $1.01x10^6$ GC/L) to effluent level (mean value $6.95x10^4$ GC/L). Despite reclaimed water of both STP accomplished water regulations regarding bacterial parameters, infective HAdV were still detected in selected samples by immunofluorescence assays (IFA).

Additionally, water samples were collected, during a year, in a third sewage treatment plant (STP3) at the entry, after secondary treatments and after tertiary treatments based on Actiflo™ technology, filtration and UV disinfection. Two locations of Llobregat River (North-East Spain) were also surveyed. The most abundant norovirus genogroup and HAdV were analyzed by q(RT)PCR. Highly variable concentrations of NoVGGII in sewage were observed during the year quantifying the highest mean values as expected in winter ($2.5x10^8$ GC/L). NoVGGII were also detected in reclaimed water in all seasons with the exception of summer, showing a reduction of 3-log from the entry to effluent level. All sewage samples tested positive for HAdV with constant mean values during the year ($3.83x10^6$ GC/L in raw sewage). Reductions of 3-log were described from entry to the effluent after tertiary treatments. NoVGGII seasonality was also identified in river water samples. HAdV was found in all analyzed samples with stable levels of prevalence and a mean value of $5.40x10^3$ GC/L.

In conclusion, NoVGGII have shown to be more prevalent than NoVGGI in the studied area East of Spain. Noroviruses and human adenoviruses have been detected in reclaimed water accomplishing current legislations and in river water. The results obtained for HAdV support their applicability as viral indicator of human fecal contamination, and have been detected in equivalent concentrations all over the year. Infectivity assays proved that infectious adenoviruses were present in reclaimed water samples by IFA. NoVGGII mean values in sewage and river water have shown to be higher than those for HAdV in winter, but lower

the rest of the year, according to this no numerical correlation should be expected between noroviruses and HAdV, however the absence of HAdV would represent lower level of fecal pollution and as a consequence lower level of risk of the presence of pathogens in water.

P10 – A Food-Borne Norovirus Outbreak during a River Cruise in Austria

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Background: Norovirus plays a major role in outbreaks on cruise ships. The virus is usually introduced on board by passengers, crew or by food items or may persist via contaminated environmental surfaces from previous cruises. Outbreak investigation is complicated due to the multitude of different countries which may be passed through during the course of the outbreak. In March 2011 a descriptive-epidemiological investigation of the Austrian Agency for Health and Food Safety hypothesized a food-borne genesis for a norovirus outbreak during a Danube river cruise between March 20 and March 24.

Methods: The following outbreak case definition was applied: A probable outbreak case was defined as a person who (1) was resident on the affected cruise ship from March 19 onwards, and (2) fell sick with diarrhoea or vomiting on March 20 at the earliest. A confirmed outbreak case was defined as a person who fulfilled criteria 1 and 2 and tested positive for norovirus. Active case finding was performed by interviewing passengers and crew members.

Food items offered at breakfast, lunch and dinner on the cruise ship at the days March 19, 20 and 21 were hypothesized as source(s) of infection. The hypothesis was tested by use of a retrospective cohort study. Norovirus detection in stool was performed by a multiplex one-tube RT-PCR. Norovirus typing was implemented by a nested multiplex RT-PCR and direct sequencing.

Results: The food-specific cohort analyses were performed day-wise for the days of interest. The analyses revealed German roast beef (served cooled), offered for dinner on March 19 with a relative risk (RR) of 1.96 (95%CI: 1.15-3.32), the scallops served for dinner, March 20 with a RR of 1.45 (95% CI: 1.01-2.08; p=0.035) and the Lamb's lettuce salad served for dinner on March 21 with a RR of 1.66 (95% CI: 1.01-2.75; p=0.043).

Molecular diagnostic resulted in different genogroups (GI and GII) and sequencing showed a multitude of different genotypes:GI.3, GI.4, GI.7, GII.4 2010, and two recombinant strains G IIb/II.13 and G II.g/G II.1.

Conclusions: The hypothesis that the reported outbreak on a cruise ship in Austria was food-borne was verified by analytical-epidemiological analysis. Furthermore, the results of the genotyping of the detected noroviruses implicate the scallops as highly likely outbreak source, confirming the analytical-epidemiological findings. Norovirus in outbreaks on cruise

ships introduced on board by passengers or crew would reflect the norovirus strain currently active in the community. The Austrian Agency for Health and Food Safety has implicated guidelines for the management of norovirus infection on cruise ships in its updated norovirus guidelines.

P11 – Foodborne Outbreak by a Recombinant Norovirus GII.g/GII.1 Strain at a University Hospital in Munich

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Noroviruses are among the most prevalent causative agents for gastroenteritis worldwide. The low infectious dose, stability in the environment, and the genetic variability enable the virus to cause outbreaks in health care facilities and other settings. Genotype II.4 has been most prevalent over the last years, evolving quickly under immune selection pressure. We characterize a GII.g/GII.1 recombinant strain causing an extended outbreak in our Munich university hospital. The outbreak spread over the hospital involving more than 100 persons within 3 days. A 2193 bp sequence covering polymerase and 1386 bp capsid gene was analyzed in 4 strains derived from different wards and the catering facility. All were virtually identical clustering around a recombinant strain described in Hungary in 2010. Subtype GII.g/GII.1 has not been detected in our hospital previously, implying a mainly immunologically naïve population both with regard to patients and hospital staff.
Compared to this Hungarian sequence, the capsid gene of our strain evolved under negative selection with 20 nucleic acid exchanges and only one resulting amino acid mutation. On the other hand the polymerase gene accumulated 18 nucleic acid mutations out of which 6 were nonsynonymous.
Our data illustrate the ability of the GII.g/GII.1 recombinant to rapidly spread within outbreaks. Thus this strain may have the potential to displace GII.4 as the most common noroviral genotype.

P12 – Molecular Characterization of Noroviruses Detected in Mediterranean Mussels (*Mytilus Galloprovincialis*) from Harvesting Areas in Slovenia

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In the years 2006-2008 the prevalence of noroviruses in shellfish from harvesting areas along the Adriatic coast of Slovenia were studied. In total, 168 samples of Mediterranean mussels (*Mytilus galloprovincialis*) were investigated. In 18 samples (10.7 %) norovirus RNA was detected by RT-PCR method amplifying a short part of the gene for RNA dependent RNA polymerase (RdRp). According the sequencing data, the most commonly detected genotype was GII.4, subtype 2006b. Beside this norovirus strain prevalent in all three harvesting areas, noroviruses of genotypes GI.2 and GII.b were also detected in samples from Debeli Rtič. The norovirus strains from mussels were compared with noroviruses detected in humans. The genetic analyses revealed close releationship among GII.4/2006b strains which is also the most prevalent genotype in humans with gastroenteritis in Slovenia.

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P13 – Occurrence of Noroviruses in Danish Mussels and Oysters

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The study: The content of Norovirus (NoV) in Danish mussels and oysters was investigated in a pilot study during 2010. The study covered 75 samples of blue mussels (*Mytilus edulis*) and 20 samples of oysters (*Ostrea edulis*). The mussels were sampled on five occasions spread over the year from a total of 17 production areas clustered in three main production regions of Denmark. The oysters were sampled in April from four production areas within the same region. The number of detectable genome copies (GC) was quantified by PCR as described elsewhere[1, 2].

Results: In total, NoV was found in 34 samples (36%) originating from 12 production areas (57% of all areas tested). In most cases (29 samples), the positive samples contained both genogroup (GG) I and II, but in one sample, only GGI was found, and in another four samples, only GGII was found. Two of the 20 samples of oysters were positive for both GGs, while all other positive samples were blue mussels. All samples were negative for Hepatitis A virus.

Eight of the 12 production areas where NoV was found were classified as being of class A based on occurrence of *E. coli*. This means that mussels and oysters from these areas with no further treatment can be placed on the market for direct human consumption.

No spatial or temporal patterns were evident as NoV was found in samples from all three regions and at five out of six sampling days. However, heavy rain falls were reported prior to two of the sampling days, which caused sewage treatment overflow and outlet to some of the production areas.

In the positive samples GGI was found in an amount corresponding to 13-2047 GC/g digestive tissue (DT), while 1-262 GC/g DT was found for GGII. It is noteworthy that no outbreak of human gastroenteritis has ever been attributed to consumption of legally harvested Danish mussels or oysters, although the GC numbers found in this study are not far beyond levels recently found in imported oysters causing outbreaks. These oysters contained 118-8891 GC/g DT GGI or 9-251 GC/g DT for GGII, but the NoV copies found may differ qualitatively from the NoV copies found in this study in regard to infectivity.

Discussion: NoV genetic material detected in mussels and oysters by PCR is not necessarily present in the form of infectious virus particles. It is thus difficult to conclude on the impact of the findings in the present study. This highlights the need for research to establish the relationship between detection of NoV in oysters by PCR and human health consequences as well as for establishing an acceptable limit for NoV in oysters as pointed out recently by EFSA[3].

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[3]EFSA Journal 2012; 10(1)2500

P14 – Human Norovirus Prevalence and Quantification in Mussels, Clams and Cockles: A One Year Study

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Viral shellfish safety continues to be a sanitary challenge. Bivalve molluscs contaminated with enteric viruses are the most common food-borne cause of gastroenteritis, with norovirus (NoV) as the main aetiological agent [1, 2]. Sanitary surveys are a useful tool to assess and control shellfish harvesting areas and recognize present or potential pollution sources [3]. Moreover, quantification techniques like real time RT-PCR (rRT-PCR) provide valuable information for the establishment of certain limit values in the legislation and how these might affect the aquaculture sector.

In this study, NoV genogroups I and II (GI and GII) were detected and quantified in a shellfish sanitary survey conducted during one full year (2011), in Galicia, NW of Spain. The samples included four bivalve mollusc species: wild and cultured mussels (*Mytilus galloprovincialis*), clams (*Ruditapes philippinarum* and *Ruditapes deccusatus*) and cockles (*Cerastoderma edule*), sampled monthly from two different localization (in the north and south of Galicia).

For virological analysis, digestive tissue (DT) was removed by dissection and homogenized in a volume of 0.1 % tryptone water (pH 7.4). After 1 h of shaking and subsequent centrifugation 1,000 x g during 5 min, RNA was extracted with the NucleoSpin RNA virus kit (Macherey-Nagel). The rRT-PCR technique with TaqMan probes was employed for viral detection, using Platinum quantitative RT-PCR Thermoscript one step system (Invitrogen). For accurate quantification, extraction and rRT-PCR efficiency controls were included in each analysis in order to not underestimate the real viral load [4].

A total of 114 samples were analyzed, from which 65 (57%) showed NoV presence. NoV GI was the most prevalent genogroup with 44 positive samples (67.7%) while NoV GII was detected in 16 samples (24.6%). Five samples (7.7%) showed mixed contamination with both genogroups. Quantification levels ranged from 10^3 to 10^9 and 10^3 to 10^6 RNA copies/g DT for GI and GII respectively. Regard to NoV prevalence as a function of the bivalve species, cultured mussels were the most contaminated with 66.7 to 72% of the samples positives depending on the sampling point, followed by wild mussels (45.4 to 66.7%), cockles (45.4 to 58.3 %) and clams (33.3 to 40 %). A clear seasonality of NoV prevalence was observed in the cold months, mainly for GII that was only detected between September and January. In addition, a relation with the regime of precipitations also was observed, with increases in NoV occurrence after abundant rainfalls.

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P15 – Norovirus GI and GII Prevalence in Bivalve Molluscs and Vegetables in Italy

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In recent years, Noroviruses (NoV) are considered the major cause of non bacterial gastroenteritis in industrialized countries, although there is still reason to believe that their number is underestimated. According to the nucleotide sequence of the NoV capsid region, they are divided into 5 genogroups (GI, GII, GIII, GIV and GV), which in turn contain several clusters or genotypes. The majority of strains infecting humans belongs to genogroup I, with 14 genotypes, and the genogroup II, with 17 genotypes (1). Transmission may occur by "person to person" contact or by consumption of contaminated water or food. In this context, bivalve molluscs and raw or ready to eat (RTE) vegetables play a special role. It is important to note that Italy is the third European producer of molluscs, after Spain and France and every year 180,000 tons of molluscs are harvested in Italy (2). Despite the epidemiological evidences, the current European legislation (EC Reg. 1441/2007) included no microbiological criteria for the control of viral contamination in foodstuffs. The availability of molecular methods based both on conventional PCR (i.e. RT-booster-PCR), or on Real-time PCR, permitted to assess the prevalence of NoV GI and GII in these types of foods.

We report data obtained from surveys carried out on bivalve molluscs collected on import sites and on markets. Moreover, two surveys were carried on to verify NoV contamination in vegetables. Of these, the first was conducted in Lombardia region (Northern Italy) on samples from the market, while the second was done in Lazio region (Central Italy) on samples from two companies, differing for their microbial stabilization technology (cryogenic removal and disinfection with halogens respectively). The total number of analysed bivalve mollusc samples was 872. The 6.4% of samples collected on markets was positive, while 29.3% of samples collected on import sites were contaminated by NoV. Vegetables samples from Lombardia region were 297, and 6 samples (2.02%) were positive for NoV: in three samples was confirmed the presence of NoV GII/4. In the monitoring carried out in Lazio region 124 samples were analysed (46 raw materials and 78 packaged RTE products analysed on their last day of shelf life). All samples were negative for Norovirus.

These data underline the need to integrate the European Community legislation on microbiological criteria for possible virus contamination in foodstuffs. Furthermore, it is important that the food business operators, both in case of import or in case of primary production, take into account the possibility of a viral contamination, and improve their HACCP programs in order to ensure that food safety is not compromised.

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P16 – Full Year Norovirus Surveillance Study on Source Water for Four Drinking Water Treatment Plants in Sweden

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Norovirus (NoV) has been indicated as the most important viral pathogen in future waterborne disease outbreaks both by the WHO in *Guidelines for Drinking-water Quality*[1] and in the Swedish Government Official Report, *Climate and vulnerability SOU 2007:60.*[2]
NoV has been the cause of several waterborne disease outbreaks in Sweden[3], and still little is known about background levels of NoV in source water. Background levels of pathogens are required to perform a reliable risk assessment, a valuable tool to improve the water treatment. Traditionally Swedish drinking water treatment plants (DWTP) were modeled to remove mainly fecal bacteria, but a greater awareness regarding protozoal and viral contamination has increased the demand for additional treatment steps.
In this study source water for four large municipal DWTPs in Sweden was sampled every two weeks for one year (September 2010 – October 2011). The sample sites included are two locations in Lake Mälaren, the third largest lake in Sweden with a very low water flow. Göta älv, a large river in western Sweden with a high flow, and Lake Ringsjön in southern Sweden with a low water flow. In total the four DWTPs provide clean drinking water to over 2,500,000 consumers.
Each sample was subjected to a three-step isolation method of virus particles. Firstly, a two-step filtration procedure using neutral and charged filter membranes was applied. Secondly, ultrafiltration using a centrifugal microconcentrator was conducted. Murine norovirus (MNV-1) was used as a process control for entire isolation procedure. Viral nucleic acid extraction was performed on the remaining retentate, followed by cDNA-synthesis using random hexamer primers. A TaqMan real-time PCR assay was used to detect and quantify concentration of NoV genogroup one (GI) and genogroup two (GII).
NoV GII was detected from early October 2010 to early April 2011 with the highest quantifiable titer in early January 2011. Scattered findings of NoV GI were detected during the same time frame. The concentrations of NoV from this study will be used as input values in a Quantitative Microbial Risk Assessment (QMRA) model to improve the quality of the simulations and thus obtaining a better understanding of how the DWTP functions during low and high load of NoV, and potential implications for public health.

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P17 – Viruses in Drinking Water

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Norovirus and rotavirus infections are triggered through faeces, either in food or drinking water, before being transmitted secondarily, especially in hospitals, old-age homes, schools and/or day-care centres.
Viruses can be detected in our waters, even in ground water, and cannot be filtered out regularly by our conventional water treatment plants. Cold water conserves contagious viruses. Apparently, the distinct seasonal waves of norovirus and rotavirus infections that appear every year strictly follow the pattern of cold temperatures in water and in water pipes. Our food has more or less the same temperature throughout the year, but not drinking water. The latter reaches its minimum temperature at the end of winter in February/March, when the sum of cold temperatures and the number of viral infections are at a maximum. Therefore, drinking water must be the abiotic medium that causes the norovirus and rotavirus infections that appear seasonally.
It is possible to filter viruses out of drinking water through ultrafiltration, without the addition of chemicals. The extra costs are only 5 Euros per person and year, which is less than 2 Euros per month for a family of four.
The chains of infection have to be recognized and interrupted by preventive healthcare strategies. Effective treatment of drinking water would reduce healthcare costs, including those caused by other infections transmitted through drinking water such as legionella, adenovirus, cryptosporidiosis, giardiasis, campylobacter, E. coli enteritis, EHEC/HUS/STEC, salmonellosis and yersiniosis. The H5N1 avian flu and the recently discovered H1N1 swine flu, that often cause vomiting and diarrhoea leading to the contamination of sewage and waters, can also be transmitted via drinking water.
An epidemiological analysis has come to the conclusion that it is highly probable that even seasonal influenza is transmitted primarily through drinking water. Science is only possible if doctrines are questioned.

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P18 – Human Norovirus Infection: An Italian Case

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Noroviruses (NoVs) are small, non-enveloped, icosahedral viruses members of *Caliciviridae* family and are a major cause of outbreaks of acute gastroenteritis world-wide.[1] NoVs have a single-strand, positive sense RNA genome of 7.5-7.7 kb in length with three *open-reading-frames* (ORFs), that encode for structural and non-structural proteins. NoVs are genetically different and have been classified into five genogroups based in homology sequence of capsid gene; NoVs GGI, GGII and GGIV have the most significance to human health.[2] These viruses are commonly identified in different foodmatrices and mussels; red fruits and vegetables are often contaminated. Recently, the central role of water as an important route of human NoVs transmission was demonstrated. NoVs are the third most frequent pathogenes for waterborne disease. Several large foodborne and waterborne outbreaks due to NoVs have been described, but there are only a few reports in which both epidemiological and environmental data have been confirmed by molecular data on the waterborne NoVs.[3]
The present work describes the epidemic case occurred in the Alps village of Malonno in Valle Trompia (Lombardy) on September 2011, when a group of 30 children between 13 and 18 years old, showed the typical symptoms of gastroenteritis during a summer camp. Three persons were also hospitalised. At the Food Safety Department of Istituto Zooprofilattico in Brescia, one faecal sample and one rectal swab from a female patient, and three water samples, collected from a drinking tube near the river of the camp, were analysed.
The faecal sample and the rectal swab were clarified in sterile distilled water (1 g in 10 ml), while water samples were filtered in acid conditions, also with $AlCl_3(6H_2O)$ 0.5 mM addition . After the RNA extraction with a commercial kit, the retrotranscription of the nucleic acid was performed and the cDNA was amplified with two Real-Time PCRs, specific for GGI e GGII NoVs genogroups. The faecal sample, the rectal swab, and one of the three water samples were positive for NoV GGI. These results were also confirmed performing a "booster PCR" giving 326 bp amplicons. Sequencing analysis of the amplified DNA, executed in Brescia and in the Istituto Superiore di Sanità in Rome, confirmed the presence of NoV GGI, strain GI.4, in all the three samples. The comparison of the GI.4 NoV sequences, showed 100% homology.
These data support the hypothesis that water could had a role in the transmission of Noroviruses to the children attending the summer camp. Water from the collecting site, probably, derived from the river and contamination could arose by pastures located upstream.

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P19 – Norovirus in Food: Effects of Technological Processes on the Tenacity and Inactivation of Human Norovirus Genogroup II

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Human norovirus (hNV) is the most common cause of nonbacterial gastroenteritis. In addition to the person-to-person transmission of hNV, indirect transmission modes via daily use and foodstuff are described. The predominant numbers of norovirus outbreaks are caused by secondary contaminations, which are often a result of hygiene deficiencies within food production, processing and preparation. In the study presented selected foods belonging to different product groups (e.g. convenience, delicatessen, meat, fruits, and vegetables) were artificially contaminated with a defined number of infective human norovirus (genogroup II). The samples were subjected to a number of processes used by the food industry for preservation and by the consumer for storage and preparation to determine the inactivation potential. After physicochemical treatment (freezing, cooling, acidification, heating) hNV was re-extracted from the food sample using an ultrafiltration-based method. The efficiency and reproducibility of the extraction was monitored by using bacteriophage MS2 as an internal process control.[1] Due to the lack of a cell culture, hNV was quantified by using monoplex one-step TaqMan real-time reverse transcription (RT)-PCR and an external standard curve based on recombinant RNA standards. In order to detect intact virus particles (with unknown infectivity), an RNase pretreatment step was carried out to avoid the problem of false-positive results caused by accessible RNA molecules originating from destroyed virus particles. Significant titer reductions were obtained with heat treatments usually applied by consumers for food preparation (baking, cooking, roasting). Generally, processes used for preservation and storage, such as cooling, freezing, acidification, and moderate heat treatments (pasteurization), appear to be insufficient to inactivate hNV within a food matrix or on the food surface. Additionally, individual food matrix-specific protective effects, rates of recovery from a product, and inhibitory effects on the PCRs were obtained in this study.[2,3] The established extraction procedure might be used for other noncultivable enteric RNA viruses that are connected to food-borne diseases.

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P20 – Effect of Acidification, Salting, Curing and Bacterial Fermentation on Potential Human Norovirus Surrogates

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Regarding possible norovirus transmission due to consumption of contaminated food, questions emerged about the effect of food processing conditions on virus infectivity. In the absence of a suitable bioassay to differentiate infectious from noninfectious virus particles research on inactivation of human noroviruses has mainly focused on studies using surrogates which can be propagate in cell culture.

In the present study* the two potential model viruses feline calicivirus (FCV) and murine norovirus (MNV) were used to examine the antiviral effect of acidification, salting, curing, and bacterial fermentation. For both viruses cell-culture assay was used to detect infectious virus particles.

FCV showed high stability when esposed to different solutions of sodium chloride (2-20 % w/v) and of D,L-lactic acid (pH 6.0-3.2) at refrigeration temperature (4 °C). At the same conditions, FCV titer decreased more markedly at 20 °C. Sodium nitrite (100, 150 and 200 ppm) did not show any antiviral effect on FCV. In studies with MNV the antiviral properties of bacterial fermentation were tested. For this, MNV was exposed to cell-free supernatants of different starter- and protective cultures used for raw sausage fermentation (lacid acid bacterias, *Staphylococcus xylosus*/*carnosus*, *Kocuria varians*) as well as to D,L-lactic acid (pH 5.8-5.2). In general, MNV showed high stability even if a low susceptibility towards lactic acid (pH<5,6) and towards the supernatant of a *Lactobacillus curvatus* strain was observed.

In summary, the present results indicate that acidification, salting, curing and bacterial fermentation may be insufficient for effective inactivation of human norovirus during food processing.

*This research was supported by the FEI e.V. Bonn (Research Association of the German Food Industry), the German Federation of Industrial Research Associations (AiF) and the Ministry of Economics and Technology. AiF project no.: 15189 BR and 16509 BR.

P21 – Stability of Released Norovirus RNA against Degradation in Selected Food Samples and against Defined Heat Treatments

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Fragments of RNA molecules can be released from non-enveloped viruses, like norovirus, by disruption of its capsid due to physicochemical treatments like freezing, heating, disinfection etc. Released RNA in samples is problematic since it causes false-positive results within real-time RT-PCR analysis and leads to an overestimation of intact virus particles in quantification experiments. It is generally accepted that RNA outside a capsid is degraded soon by omnipresent environmental RNase activity and that RNA is generally not very stable against heat treatments. In the present study the stability of isolated RNA from human norovirus (GGII) against degradation under environmental conditions in food samples by native RNase activity and its stability against heat under defined laboratory conditions without RNase activity was tested. Therefore, defined amounts of hNV RNA were added to two different food samples, untreated drinking water and pasteurized tomato sauce. The samples and control samples (positive and negative) were incubated for 24 h. Subsequently, the amount of RNA was analysed with qualitative real-time RT-PCR. In unsterile drinking water a RNA degradation was detectable but not complete after 24 h of incubation. The degradation in tomato sauce was even less compared to the water samples. Native RNase activity was, under the conditions tested, not sufficient to completely degrade greater amounts of RNA, which are analogous to those amounts theoretical being released in hNV spiking experiments (~$4x10^9$ particles/ml). For the heat experiments sterile PBS (phosphate buffered saline)-buffer samples were spiked with defined amounts of isolated hNV RNA. The independent samples were incubated for different time periods at 95 °C. Subsequently, the amount of RNA was analyzed using qualitative real-time RT-PCRs. Up to 30 min the amount of RNA was constant. A complete elimination of detectable RNA fragments was observed between 180 min and 240 min at 95 °C. The results show that RNA fragments detectable with real-time RT-PCR are notable heat stable. Neither native RNase activity in environmental samples nor heat treatments (< 180 min at 95 °C) are sufficient to completely eliminate greater amounts of RNA. For reliable quantitative real-time RT-PCR detection of intact RNA virus particles a preliminary RNase treatment step is shown to be obligatory.

P22 – Specific Pathogen-Free Piglets Do Not Replicate Human SaV

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Sapoviruses (SaVs) are enteric virus that can infect human and animals. Human SaV are classified in four genetic clusters, namely genogroup (G) I, II, IV and V. Porcine SaV is classified only in GIII, but other genogroups are proposed: GVI, GVII, and GVIII (Farkas et al., 2004; Martella et al., 2008). The genetic identity between human and porcine SaV strains can be high, suggesting the zoonotic potential of SaV (Bank-Wolf et al., 2009). In the present study, specific pathogen-free piglets (SPF) were inoculated with porcine and human SaV strains to compare the dynamics of infection produced by virus strains from different host origin. The experiment was performed in a BSL-2+ facility, and was approved by the ethics committee of the Animal Sciences Group, Wageningen University and Research Centre. Inocula were prepared with 10% fecal suspensions in MEM with one sample positive for SaV GVII and another for SaV GI.2 (Parkville-like strain). Three groups of SPF piglets were inoculated orally and intravenously, at 6 days of age with 2 mL of the inocula. Group T02 and T04 were composed of four piglets each and were inoculated with SaV GVII and GI.2, respectively. The control group was inoculated with MEM and was composed of five piglets. Fecal samples were collected daily directly from the pig rectum. At the necropsy, performed at 6 or 7 days post inoculation (dpi), fragments of intestine (duodenum, jejunum, ileum, and colon) were collected and stored at -80°C. RNA extraction from fecal and organ (macerated) suspensions was performed using the High Pure RNA Isolation Kit (Roche, Mannheim, Germany) according to the manufacturer instructions. To test the samples from T02 (porcine SaV GVII) a TaqMan qPCR were developed with primers pSGVIII-fwd (5' – ATT GAC ATY TTG GGT GAG TTY ATT GA – 3') and pSGVII-rev (5' – GGC ART GAG ATG TCA AAY ACA AG – 3'), and the probe pbSGVII (5' – ACG ATA CAA TCT GTG CTC AA – 3'). For group T04 (human SaV) the protocol described by Oka et al. (2006) was employed. Samples from the control group were tested with both protocols. The four piglets in each group (T02 and T04) showed the same pattern of virus shedding. The viral load in fecal samples from all piglets in T02 was constantly increasing until the day of euthanasia, which clearly shows viral replication. In the qPCR of the intestine fragments, jejunum and duodenum presented higher viral load then ileum and colon, suggesting that the proximal small intestine is site for virus replication. In opposite to T02, in group T04, inoculated with human SaV, the SPF piglets showed decrease in viral load and only in the initial 1 and 2 dpi. No virus was detected by qPCR in the intestine fragments. These data together, indicates a non productive infection. In the control group one animal was positive for porcine SaV at 6 dpi. The other samples and the intestine fragments were negative for porcine and human SaV. In summary, the present study

indicates that the swine is not a reservoir for SaV GI.2, the most commonly human SaV detected in outbreaks (Svraka et al., 2010). Furthermore, since pigs can not replicate this strain, it is unlikely that recombination events involving human and porcine SaV can occur in pigs.

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P23 – Characterization of the Specificity of Monoclonal Antibodies against Norovirus Genotype GII.4

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Introduction: Noroviruses are the leading cause of acute viral gastroenteritis in humans worldwide. Over the past decade most reported norovirus outbreaks and epidemics have been caused by variants of norovirus GII.4 genotype. The P2 subdomain of the major capsid protein of GII.4 strains evolves rapidly, resulting in new epidemic strains with altered antigenicity and histo-blood group antigen (HBGA) ligand binding properties.
Aims: To produce monoclonal antibodies against norovirus genotype GII.4 and to evaluate their epitope specifity. Our main goal is to develop broadly-reactive antibodies against different norovirus genotypes.
Methods: Monoclonal antibodies (mAb) were raised against norovirus GII.4 genotype (2006b variant) VLPs. Mice were immunized with GII.4-2006b variant VLPs and hybridomas were selected by ELISA using norovirus genotype GI.5 VLPs as the antigen. Some of the selected mAbs recognized norovirus strains in clinical samples. The 4C5C10 IgG mAb showed the highest reactivity both by ELISA and IFA. This mAb recognizes GII.4 VP1 in ELISA, but very weakly by Western blot under reducing conditions, suggesting that the interaction between this mAb and VP1 is conformation-dependent. The epitope recognized by this mAb was analysed by phage display, using the Ph.D.™-C7C phage display peptide library (New England

Biolabs). After three rounds of selection, 19 clones were sequenced, yielding a 30 amino acid consensus sequence.

Results: The conformational epitope recognized by mAb 4C5C10 has been identified in the crystal structure of the VA387 VP1 protein by using PepSurf in the Pepitope server. This epitope is located within the P2 domain in the region involved in binding to HBGAs. In this regard, GII.4-2006b variant is particularly interesting, because it binds to non-secretor saliva samples. The 4C5C10 mAb reacts weakly in ELISA withGII.4-2004 variant VLPs, but does not recognize genotype GII.3 VLPs. MAbs raised againts GII.4-1999 (anti-v0) do not recognize GII.4-2006b VLPs, whereas other mAbs produced against GII.4-2004 (anti-v2) show high reactivity with GII.4-2006b. This observation suggests close antigenicity between GII.4-2004 and GII.4-2006b variants.

P24 – Intervention into Norovirus-Host Cell Attachment: Studies with NMR and SPR

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Noroviruses are a prominent example for carbohydrate-mediated host cell attachment of non-enveloped viruses. The recognition of so called histo-blood group antigens (HBGAs) located on the surface of the gastrointestinal epithelium has been identified as crucial step in the infection process. No therapeutic treatment or vaccination strategy has been developed so far and tissue or cell culture models exhibit limited availability. Thus, in vitro experiments utilizing virus like particles (VLPs) and synthetic HBGAs serve as model system to study virus-host cell attachment.

We use ligand-based NMR experiments such as Saturation Transfer Difference (STD) NMR as well as Surface Plasmon Resonance (SPR) experiments to gain important information on virus-host-cell interaction at atomic resolution. Thereby a comprehensive picture of the natural binding pattern of a GII.4 norovirus strain could be obtained. A strong preference for fucosylated oligosaccharides was found as previously suggested by multiple sequence alignment of GII.4 capsid proteins. This information together with hits from screening of small fragment and virtual compound libraries was used for the design of fucosylated inhibitors that were further characterized by NMR and SPR. Multivalent compounds were identified as efficient inhibitors that serve as prototype entry-inhibitors against a norovirus infection. The poster focuses on the determination of binding affinities to wildtype and mutant VLPs based on NMR and SPR.

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P25 – Lewis Histo-Blood Group α1,3/α1,4 Fucose Residues May Both Mediate Binding to GII.4 Noroviruses

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Human noroviruses cause recurrent epidemics of gastroenteritis known to be dominated by the highly prevalent and clinically important GII.4 genotype which recognizes human ABH histo-blood group antigens (HBGAs) as receptors or attachment factors (Donaldson et al., 2010; Hutson et al., 2004). There are, however, conflicting reports on the recognition of the Lewis antigens by GII.4 noroviruses (de Rougemont et al., 2011; Fiege et al., 2011; Huang et al., 2005; Rydell et al., 2009). In the present work we have investigated the possibilities of the Lewis α1,3/α1,4 fucoses as mediators of binding of Lewis antigens to GII.4 noroviruses. The study was carried out with molecular dynamics (MD) simulations of Lewis type-1 and type-2 chain HBGAs in complex with VA387 P-domain dimers for at least 5 ns in explicit water. Stable trajectories were obtained with the Lewis α1,3/α1,4 fucose residues tightly bound in the "secretor α1,2 fucose" binding site of VA387.
Based on the computer simulations, we suggest two possible receptor binding modes for Lewis HBGAs: the "secretor pose" with secretor Fucα1,2 in the binding site and the "Lewis pose" with Lewis Fucα1,3/α1,4 residues in the binding site and in the same orientation as previously reported by crystallographic data (Cao et al., 2007). The results can explain the interactions of GII.4 norovirus with Le^x and SLe^x structures. Moreover, the present modeling suggests binding of complex branched polysaccharides, with the Lewis antigens at the non-reducing end, to P-domain dimers of GII.4 strains. The results are important both for understanding the binding of Lewis antigens to GII.4 noroviruses at the molecular level and for the *in-silico* design of antiviral therapeutics.

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P26 – Development of Coumarin–Thiopurine Ribofuranoside Conjugates with Anti-Viral Activities

Susan Shwu-Chen Tsay[†], Xin-Hong Liu[†], Ching-Jui Hsu[†], Shih-wei Lo[†], Ming-Yang Gao[†], Shu-Yu Lin[‡], Johan Neyts[§], Pieter Leyssen[§] & Reuben Jih-Ru Hwu[†,‡]

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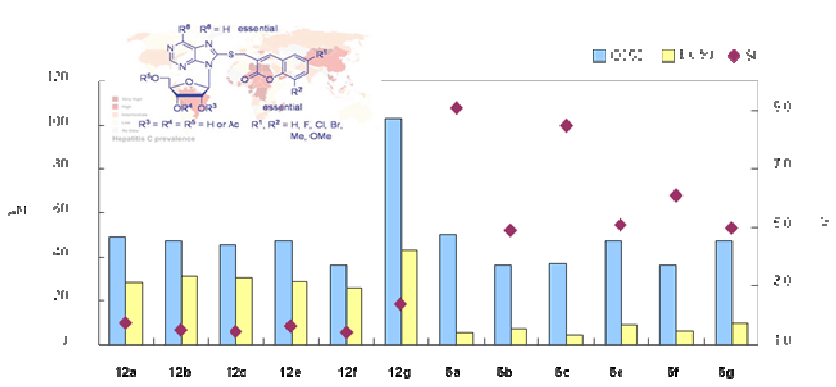
In total 26 new conjugated compounds were synthesized through the chemical coupling of various 9-(β-D-ribofuranosyl)purine-8-thiones with 3-(chloromethyl)coumarins bearing various substituent, including F, Cl, Br, Me, and OMe. A methylenethio linker was used to connect these two kinds of derivatives. Their activities against various viruses were tested. Among them, three conjugates in the family of 8-(coumarin-3′-yl)methylthio-9-(β-D-ribofuranos-1″-yl)purine were found to possess an appealing ability to inhibit HCV replication with EC_{50} between 5.5 and 6.6 μM and EC_{90} of ~20 μM.



Given these data, a structure–activity relationship was established. It is an essential for the coumarin moiety present in the conjugated compounds to exhibit antiviral activity. Lack of the sulfur atom-containing linkage led to poor biological activities. These guidelines are of value for medicinal scientists to design and synthesize new conjugated compounds as potential anti-viral leads in the control of emerging and neglected diseases caused by RNA viruses including norovirus.

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P27 – Force Microscopy – a Novel Tool to Elucidate Nanomechanics and Functional Properties of Norovirus-like Particles

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Norovirus-like particles (NLPs), were imaged with Atomic Force Microscopy. The mechanical stability of the VLPs was probed by nanoindentation at pH values ranging from 2-10. This range includes pH values of the natural environment during the life cycle of noroviruses. The resistivity of VLPs toward indentation was constant at acidic and neutral pH. The young modulus was of the order of 30 MPa (Mega Pascals). At basic pH the compliance of the capsid increased along with an increase of the diameter. This specific pH-dependent mechanical response of the capsid may have a relation to mechanisms controlling uptake and release of the RNA during infection. Consecutive indentations with pressures up to 300 bars demonstrated the ability of the capsids to fully recover from deformations comparable with the size of the capsid. The capsids can be viewed as nanocontainers with an inbuilt self-repair mechanism. At pH 10 the capsids lost their stability and were irreversibly destroyed after one single indentation. Force spectroscopy could furthermore contribute to test the strength of Norovirus capsids under increasingly applied osmotic pressures. The mechanical characterization of these protein shells under different environmental conditions could potentiate their application as drug delivery systems. Future applications of this technique through chemical functionalization methods will allow probing single virus-receptor interactions, fundamental for vaccine development.

P28 – Structure-Based Inhibition of Norovirus RNA-Dependent RNA-Polymerases

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Caliciviridae are RNA viruses with a single-stranded positively-oriented polyadenylated genome, responsible for a broad spectrum of diseases such as acute gastroenteritis in humans (NV and SV), hemorrhagic disease in rabbits (RHDV), upper airway infection in cats (feline calicivirus), and also lethal encephalitis in immune deficient mice (MNV). Recently, analyses on the structures and functionalities of the RNA-dependent RNA-polymerase (RdRp) from several Caliciviruses have been reported. The RdRp domains have been shown to share the typical "right hand" overall structure, with differences localized in the C-terminus in the palm domain. Moreover, the structure of hNV bound to template and primer RNA provided a high resolution description of the protein during RNA elongation. Therefore the RdRp is predicted to play a key role in genome replication, as well as in synthesis and amplification of additional subgenomic RNA.
Starting from the crystal structures of human norovirus RdRp (hNV), we performed an in silico docking search to identify synthetic compounds with predicted high affinity for the enzyme active site. The best ranked candidates were tested *in vitro* on murine norovirus (MNV) and hNV RdRp to assay their inhibition of RNA polymerization. The results of such combined computational and experimental screening approach led to the identification of two high-potency inhibitors: Suramin and NF023, both symmetric divalent molecules hosting two naphthalene-trisulfonic acid heads. The crystal structure of MNV RdRp is reported alone, and in the presence of the two identified inhibitors. Both inhibitory molecules occupy the same RdRp site, between the fingers and thumb domains, with one inhibitor head close to residue 42 and to the protein active site. To further validate the structural results, we mutated Trp42 to Ala in MNV RdRp, and the corresponding residue (*i.e.* Tyr42 to Ala) in hNV RdRp, showing decrease of inhibitory potency of the two compounds for both the mutated proteins.

P29 – Self-Assembly of Norovirus-like Particle by Expression of Codon-Usage Optimized Capsid Protein VP1 in *E.coli*

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Norovirus (NV) is a positive stranded RNA virus and is responsible for the most cases of viral gastroenteritis worldwide. The norovirus capsid consists of 180 copies of the 58-kDa VP1. Recombinant expression of the VP1 in baculovirus system leads to a spontaneous formation to virus-like particle (VLP). Because of the lack of a suitable cell culture system, VLPs are e.g. useful to study the immunogenicity of NV and host-receptor interaction. Until now self-assembly into VLPs could not be detected after expression of the capsid protein in E.coli.
The aim of this study was to verify if a codon-usage optimization of the VP1 for the improved expression in *E.coli* affects the formation of Norovirus-like Particle. After a phylogenetic analysis of 507 full-length-sequences of the capsid protein from GenBank (NCBI), we chose the seven most abundant genogroups or cluster for a codon-usage optimized synthesis of the capsid sequences. The optimized VP1 were expressed in *E.coli* by fermentation and purified by HisTrap and gel filtration chromatography, yielding VLPs of 0,2 mg to 5,5 mg per 2L *E.coli* culture. Self-assembly of Norovirus-like particle was verified by transmission electron microscopy. The production of VLPs in *E.coli* is a simple and effective alternative to the expensive and time-extensive expression in baculovirus-system.

P30 – Attempts to Propagate Human Noroviruses in Three-Dimensional Cell Cultures

Sayaka Takanashi[1], *Linda J. Saif*[1], *John H. Hughes*[2], *Tea Meulia*[3], *Kwonil Jung*[1], *Kelly Scheuer*[1] *& Qiuhong Wang*[1]

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Although four decades since the discovery of human noroviruses (HuNoVs), their replication mechanisms are largely undefined due to lack of a cell culture system for their routine in vitro propagation. Two publications reported cultivation of HuNoV using intestinal cells that differentiated to form microvilli under three-dimensional (3D) conditions (Straub et al 2007; 2011). We expanded this observation using the same cell lines and 2 HuNoV strains, including an emerging genotype. Human intestinal cell lines Caco-2 and INT-407 cells were seeded into rotating wall vessels (RWVs) together with microcarrier beads and maintained for 21-28 days until cell aggregates (2-3 mm in diameter) formed as an intestinal organoid model. HuNoVs genogroup (G)II.4 and GII.12 strains were inoculated directly into the RWV

or onto cell culture plates containing formed aggregates at ratios of 3-4 $\log_{10}$ genomic equivalents/cell. Morphological examination of the 3D cultures demonstrated that these two types of intestinal cells efficiently adhered to the microcarrier beads and that they assembled to form 3D tissue-like aggregates containing 5-10 beads per aggregate. Light and electron microscopic observations revealed that they differentiated to form apical microvilli that resemble those of native intestinal epithelial cells. However, the HuNoV RNA titers in supernatants and cell lysate samples, tested by real-time RT-PCR, did not increase when tested up to 120 hours-post-inoculation and no viral antigen was observed in the paraffin-embedded aggregates by immunofluorescence microscopy using guinea pig antisera against the corresponding virus-like particles. Collectively, we performed twelve experimental replicates using these two cell lines and different microcarrier beads and culture supplements (bile acids, simvastatin, and intestinal content preparations from germfree pigs) that were effective for replication of other enteric viruses. An inoculation trial using GI.1/Norwalk HuNoV as was used by Straub is currently underway.

P31 – Small Inhibitor Leads Versus Emerging and Neglected RNA Viruses (SILVER)

The SILVER Consortium. Coordinators: Ernest A. Gould & Jean-Louis Romette

Unité des Virus Emergents, UMR 190 Université de la Méditerranée-IRD), ESIL, Case 925, 163 Avenue de Luminy, 13288 Marseille Cedex09, France

This project aims to identify **S**mall molecule **I**nhibitor **L**eads **V**ersus **E**merging and neglected **R**NA viruses (SILVER). It focuses its activities on selected medically important RNA viruses for which the development of drugs is considered essential (Dengue-, entero- and paramyxoviruses), whereas other relatively neglected and/or emerging RNA viruses are explored to identify the most promising viral protein targets and antiviral compounds. A pipeline strategy has been developed to enable the inclusion in SILVER of viruses at all levels of existing knowledge.
The Consortium is composed of the following 23 participants:

1	Université de la Méditerranée, Aix-Marseille II a. EPV b. AFMB	France
2	University of Lübeck	Germany
3	Katholieke Universiteit Leuven a. Laboratory of Virology b. CISTIM	Belgium
4	Leiden University Medical Centre	The Nethlds
5	Erasmus University Medical Centre	The Nethlds
6	Institut Pasteur	France
7	Universitätsklinikum Heidelberg	Germany

8	Radboud University Medical Centre	The Nethlds
9	Riboxx GmbH	Germany
10	University of Oxford	UK
11	CNRS	France
12	Consejo Superior de Investigaciones Científicas	Spain
13	Global Phasing Ltd.	UK
14	Pike Pharma	Switzerland
15	University of Innsbruck	Austria
16	University of Hamburg	Germany
17	Shanghai Institute of Materia Medica	China
18	Korea Research Institute of Chemical Technology	Korea
19	National Central University	Taiwan
20	Univesita degli studi di Milano	Italy
21	University of Leipzig	Germany

Targets for potential drugs include infectious virus, structurally characterised viral enzymes and other proteins. Leads for currently available antiviral drugs have been identified by screening compound libraries in virus-infected cell culture systems and *in vitro* assays using purified viral enzymes. Selective inhibitors of viral replication have also been (and are being) derived using detailed structural knowledge of viral proteins and structure-based drug design. Hits are being assayed using individual viral protein targets and replicative proteins in complex with viral RNA.The potential protective activity of the most potent inhibitors that have a favourable (*in vitro*) ADME-tox profile, will be assessed in relevant infection models in animals. Licenses on promising compounds or compound classes will be presented to the interested pharmaceutical industry and will provide a multidisciplinary framework for rapid knowledge-based response to emerging infections.

P32 – European Viral Archives (EVA)

Coordinator: Jean-Louis Romette

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The core of the European Viral Archives is composed by the following participants:

	Participant organisation name	**Short name**	**Country**
1.	Institut de Recherche pour le Développement	IRD	France
2.	Veterinary Laboratories Agency	VLA	UK
3.	Bernhard-Nocht-Institut für Tropenmedizin	BNI	Germany
4.	Universitätsklinikum Bonn	UKB	Germany
5.	Health Protection Agency	HPA	UK
6.	Université de Genève	UNIGE	Switzerland
7.	Univerza v Ljubljani	UL	Slovenia
8.	Institute of Virology, Slovak Academy of Sciences	IVSAS	Slovakia
9.	Université de la Méditerranée	UNIVMED	France

Overall objective:

The overall objective is to create and mobilise a European network of high calibre centres with the appropriate expertise, to collect, amplify, characterise, standardise, authenticate, distribute and track mammalian and other exotic viruses. The network will also produce associated reagents on demand, to laboratories throughout Europe and also worldwide. The network of EVA laboratories represents an extensive range of virological disciplines and currently holds approximately 50% of the 500 recognised species within the EVA collection. EVA will initially develop its platform on the basis of selected European laboratories that have already accumulated specialised virus collections and the expertise to take these to the state-of-the-art level through integration into EVA. EVA will also link up with other network-based virus-associated programmes that exist globally. However, looking further ahead, EVA was conceived ultimately to be an open entity aiming at **"developing synergies and complementarity capabilities in such a way as to offer an improved access to researchers"**.

EVA network extends beyond the EC through association with non EU collections:

	Participant organisation name	**Short name**	**Country**
10	OnderstepoortVeterinary Institute	ARC-OVI	South Africa
11	Research Institute of Influenza	RII	Russia

12.	Chumakov Institute of Poliomyelitis & Viral Encephalitides	IPVE	Russia
13	Biomedical Center		Russia
14	Mechnikov Research Institute	HPA	Russia
15	Ivanovsky Institute of Virology	IIV	Russia
16	Center for Molecular Diagnostics	CMD	Russia
17	St Petersburg Pasteur Institute	IP	Russia

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