PROGRAMME AND ABSTRACT BOOK

SIXTH EUROPEAN ROTAVIRUS MEETING

17 - 20 May, 2015
DIJON, FRANCE
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WELCOME

Dear Colleagues,

It is my great pleasure to welcome you to the 6th European Rotavirus Biology Meeting (ERBM) being held in Dijon, France. This 6th ERBM follows on from a successful series of international rotavirus meetings that began in Paris (France) 2005 and continued in Stockholm (Sweden) 2007, at Loch Lomond (Scotland) 2009, in Reggio Calabria (Italy) 2011 and in Valencia (Spain) in 2013.

These international meetings provide a forum for scientists who are actively involved in all aspects of rotavirus research. The aim is to stimulate the exchange of information and promote collaborations in all aspects of rotavirus research. These goals are supported by regular scientific meetings at which the participation and contributions of young researchers are particularly encouraged. We have tried to present state-of-the-art research from different disciplines, encompassing basic virology, pathogenesis, human and animal virology, molecular epidemiology and measures of prevention. This meeting will introduce some new and very exciting developments in rotavirus biology which are likely to inspire new research in the years to come.

The significance of rotavirus research is reflected in the number of participants at this meeting. Over 100 participants originating from 29 countries spread over 5 continents (Europe, Africa, North and South America, Asia and Oceania). I am sure you will find the program interesting, and invite you all to join in lively discussions.

The 6th ERBM will take place in Dijon in Burgundy. So, besides the exciting scientific exchanges opened towards the future, this meeting will be an opportunity "to get a taste" of Dijon, its history and heritage, its renowned gastronomy and, of course, the Burgundy vineyards.

On behalf of the 6th ERBM Organizing Committee

Pierre POTHIER
University of Burgundy,
Dijon, France
COMMITTEES

The 6th ERBM organizing and scientific committee

- Javier BUESA (University of Valencia, Spain)
- Ulrich DESSELBERGER (University of Cambridge, UK)
- Miren ITURRIZA-GÓMARA (University of Liverpool, UK)
- Pierre POTHIER (University of Burgundy, Dijon, France)
- Franco Maria RUGGERI (Istituto Superiore di Sanità, Rome, Italy)
- Lennart SVENSSON (University of Linköping, Sweden)

ERBM GRANT Awardees

6th ERBM grants

- BIALOWAS Sonja (Sweden)
- BONKOUNGOU Isidore (Burkina Faso)
- DE LORENZO Giuditta (Italy)
- DELOGU Roberto (Italy)
- HAGBOM Marie (Sweden)
- HUNGERFORD Dan (United Kingdom)
- IANIRO Giovanni (Italy)
- ISTRATE Claudia (Portugal)
- LUCHS Adriana (Brazil)
- MARQUES DA SILVA Marcelle (Spain)
- NYAGA Martin (South Africa)
- THEUNS Sebasien (Belgium)
- VIZZI Esmeralda (Venezuela)
SPONSORSHIP

We are grateful to the following sponsors for their support to the meeting

Société Française de Microbiologie
Sanofi Pasteur MSD
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LOCATION OF THE MEETING

Holiday Inn Dijon Toison d’Or
Parc de la Toison d’Or, 1 Place Marie de Bourgogne, 21000 DIJON – France,
Phone: +33 (0)3 80 60 46 00

A bit of history on this name “Toison d’Or”:
The name of this Dijon’s area, “Toison d’Or”, is in reference to the “Order of the Golden Fleece”
created in 1430 by Philip the Good, Duke of Burgundy, and based on the knighthood's values and the
myth of Jason.

SOCIAL PROGRAMME

Sunday, 17 May:

20:00 to 22:00 Welcome dinner at the Holiday Inn hotel. This dinner will be served as a buffet to
allow participants to settle here whatever the time of their arrival

Monday, 18 May:

18:00 to 18:30 Depature to the City Center by bus
18:45 – 20:00 Visit and aperitif in Dijon City Hall
20:15 Dinner at a restaurant in the historic center of the city:
“La Maison Millière” or “Le Petit Roi de la Lune”

Aperitif and dinner in Dijon city center, a short stroll in the past.
Dijon’s historic heritage is extremely rich: the quantity, density and quality of the houses and mansions
in the historic center place Dijon among the richest architectural cities of France. We will have time for a
short visit to the historic center on Monday evening, and there will be an aperitif in the “Salle de Flore”
in the palace of the Dukes and the States of Burgundy. In this part of the palace, extended by the
Governors of Burgundy in the 17th and 18th centuries, we can admire in the courtyard the different
styles of these centuries, the Louis the XIVth, Louis the XVth and Louis the XVIth facades and the Gabriel
staircase. In front of this Palace, on the “Place de la Liberation” (former “Place Royale”) we will have an
overview of these building and the square design by Jules Hardouin-Mansard, the King’s first architect.
Behind and literally “wrapped” within the classical style building, we will be able to see the former ducal
palace and the “Tour de la Terrasse” which dates back to the Middle Ages and shows the power of the
Great Dukes of the Occident. We will continue our stroll through the city’s medieval streets to our
restaurants for the evening meal. Our restaurants are housed in half-timbered buildings dating back to
the fifteenth century.

Tuesday, 19 May:

16:00 Depature to Beaune through the Burgundy vineyards
17:15 – 18:30 Visit of the Hotel Dieu in Beaune or a cellar “The Marché aux Vins”

The Burgundy vineyard and the” Climats”
The term “climats” designates each plot or group of plots of vines, which benefit from specific geological
and climatic conditions. Thanks to the work of vigneron and their helpers and the two main grape
Varieties (Pinot Noir for red wines and Chardonnay for white wines) an exceptional hierarchy of internationally famous “crus” have been developed. On our trip to Beaune, we will travel through the prestigious vineyards of the Côte de Nuits: Marsannay, Fixin and then Gevrey-Chambertin and its “Grands Crus” such as Chambertin, Charmes-Chambertin, Griotte-, Latricières-Chambertin...

We then leave the “route des Grands Crus” in the village of Vougeot, with, on our right, the “Clos Vougeot” - another Grand Cru - and its Château, formerly the winery of the monks of Cîteaux Abbey. If we have enough time, we will stop in Vosne-Romanée to breathe in the atmosphere of its prestigious “Grands Crus” such as La Tâche, La Romanée and … the Romanée-Conti, the most prestigious and among the most expensive wines in the world.

After this stroll through the vines, your taste buds may want to practice tasting Burgundy wine. **We will visit a cellar - the “Marché aux Vins” - or discover the rich historic heritage of the Dukes of Burgundy by visiting the “Hôtel Dieu” of Beaune.** The choice will be up to you; unfortunately, the time available does not allow us to do both tours.

**The “Hôtel-Dieu”of Beaune:**
The “Hôtel-Dieu” of Beaune (or the “Hospices de Beaune”) bears witness to the rich cultural history when the power of the Duke of Burgundy stretched from Burgundy to Flanders and the Netherlands. Founded in 1443 by Nicolas Rolin, chancellor of Philippe le Bon, this jewel of high gothic architecture, illustrates the strong bond between Burgundy and Flanders. Its polychrome roofs and the golden colours of the altar piece of the Last Judgement by Rogier Van der Weyden have made it famous. But this medieval hospital also hides other treasures: the great "Salle des Pôvres" with its richly sculpted and painted ceiling, the gothic chapel, the kitchen with an automated rotisserie and the pharmacy with its collection of pewter and earthenware.

**Cellar visit and wine tasting at the “Marché aux Vins”**.
Right in the heart of the town facing the famous Hospices de Beaune the Marché aux Vins is sited in the former Cordeliers church. This Franciscan church, which was dedicated to Saint Bernadin, was bought and restored in 1977 and today houses the Marché aux Vins. Set in this beautiful and exceptional architectural environment, the Marché aux Vins offers you the opportunity to discover its cellar with a guide and taste a selection of five Burgundian wines.

**19:00 Conference dinner in the « Château de Gilly”**

**Conference dinner:**
Our "conference dinner" will be held at the Château de Gilly – the former residence of the Abbots of Citeaux - located close to the Chateau du Clos de Vougeot. This is now a castle-hotel, and has preserved its authentic Fourteenth and Sixteenth Century character, reflected in its moats, French-style gardens and various rooms.

We will have dinner in the “Salle des Tapisseries” on the first floor of the old granary of the castle. It owes its name to tapestries, canvas reproductions of the Indies, which adorn the walls, dressing the warm tones of the stone walls. Beams and high ceilings are also remarkable. The restaurant serves dishes created by Chef Jean-Alain Poitevin, starting with an egg poached in red wine sauce, a typical Burgundian dish. Bon appétit.
PROGRAMME

Sunday, 17 May
18:00 – 20:00  Registration at the Hotel Main Hall
20:00 – 22:00  Welcome dinner

Monday, 18 May
8:00 – 8:30  Registration at the room Maranello, 1st floor
8:30 – 8:40  Welcome and opening the meeting
8:40 to 9:30  Keynote conference:
Harry GREENBERG (Stanford, USA)
Immune responses and protective mechanisms to rotavirus infection
Chairman: Lennart SVENSSON

9:30 to 10:20  Session I. Structure-function, replication and assembly
Chair: Ulrich DESSELBERGER and Oscar R. BURRONE

Oral 01  A conserved translation regulatory motif (TRM) in the 5’UTRs of rotavirus genome segments
Giuditta DE LORENZO (Trieste, Italy)

Oral 02  Early events in viroplasms formation require association with the molecular motor dynein
Catherine EICHWALD (Zurich, Switzerland)

Oral 03  Generation of an avian/mammalian rotavirus reassortant using a helpervirus-dependent reverse genetics system
Reimar JOHNE (Berlin, Germany)

Oral 04  Characterization of the cell cycle arrest induced by rotavirus
Catherine EICHWALD (Zurich, Switzerland)

(Short) & P-01  Characterization of the cell cycle arrest induced by rotavirus
Catherine EICHWALD (Zurich, Switzerland)

10:25 - 10:45  Coffee break in the exhibition and poster areas

10:45 to 12:00  Session IIA. Virus-cell and virus-gut interactions
Chair: Lennart SVENSSON and Germain TRUGNAN

Oral 05  The cholinergic anti-inflammatory pathway attenuates inflammation during rotavirus infection
Marie HAGBOM (Linköping, Sweden)

Oral 06  Intracellularly expressed NSP4 of rotavirus stimulates serotonin (5-HT) release from human
enterochromaffin cells
Sonja BIALOWAS (Linköping, Sweden)

Oral 07  Rotavirus infection induces alternative splicing of the stress-regulated transcription factor XBP1
Didier PONCET (Gif-sur-Yvette, France)

Oral 08  Selective activation of the unfolded protein response by viral complexes is essential for efficient
rotavirus production
Lan Trang VU (Paris, France)

Oral 09  Rotavirus morphogenesis doesn’t require autophagy but blocks both its initiation and fusion
steps in intestinal epithelial Caco-2 cells
Germain TRUGNAN (Paris, France)

12:00 – 13:00  Visit the poster presentations
13:00 - 14:30  Lunch buffet
14:30 to 15:15  Session IIb. Virus-cell and virus-gut interactions
Chairled by Lennart SVENSSON and Germain TRUGNAN

Oral 10  Role of histo-blood group antigens in human RVA infection
Jacques LE PENDU (Nantes, France)

Oral 11  Relationship between rotavirus infection and blood group antigens
Gaël BELLIO (Dijon, France)

Oral 12  Human rotavirus usage of glycans as cellular receptors
Barbara COULSON (Melbourne, Australia)

15:15 - 15:45  Coffee break in the exhibition and poster areas

15:45 to 16:45  Session III: Immune responses - immunopathological diseases
Chairied by Harry GREENBERG and Jacques LE PENDU

Oral 13  Rotavirus NSP1 down-regulates levels of multiple interferon receptors on intestinal epithelial cells
Adrish SEN (Stanford, USA)

Oral 14  Acceleration of type 1 diabetes development by rotavirus is associated with type 1 interferon-dependent responses in regional lymph nodes
Barbara COULSON (Melbourne, Australia)

Oral 15  In vivo and in vitro adjuvant properties of rotavirus VP6 protein
Vesna BLAZEVIC (Tampere, Finland)

Oral 16  Competitive homing of rotavirus-specific memory B cells towards the gut-associated lymphoid tissues
Davide AGNELLO (Dijon, France)

17:00 – 18:00  Visit the poster presentations

18:00 to 18:30  Departure to the Downtown by buses
18:45 to 20:00  Visit and aperitif in Dijon City Hall
20:15  Dinner in the restaurant “La Maison Millière” or “Le Petit Roi de la Lune”

Tuesday, 19 May

8:30 to 10:00  Session IV Rotaviruses and animal infections
Chairled by Ken MELLITS and Helen O’SHEA

Oral 17  Phylogenetic evolution of animal rotavirus B
Tohru SUZUKI (Tsukuba, Japan)

Oral 18  Metagenomic identification of group A and B rotaviruses in faeces of urban wild rats
Reimar JOHN (Berlin, Germany)

Oral 19  Complete genome characterization of recent and ancient Belgian pig group A rotaviruses and assessment of their evolutionary relationship with human rotaviruses
Sebastiaan THEUNS (Ghent, Belgium)

Oral 20  A vaccine strategy against porcine rotavirus using cell lines deficient for innate antiviral mechanisms
Nathan MEADE (Nottingham, United Kingdom)

Oral 21  Epidemiological and phylogenetic analysis of avian rotaviruses in Italy
(Short) & P-04  Chiara BUSI (Brescia, Italy)
Session V: Environment, molecular epidemiology and animal to human transmission
Chair by Franco RUGGERI and Jose Paulo LEITE

Oral 24
Human rotavirus removal in a membrane bioreactor wastewater treatment process
Takayuki MIURA (Nantes, France)

Oral 25
Mining and modelling; human and animal rotavirus epidemiology in Ireland
Helen O’SHEA (Cork, Ireland)

Oral 26
Whole genome detection of rotavirus mixed infections in human, porcine and bovine samples co-infected with various rotavirus strains collected from sub-Saharan Africa.
Martin NYAGA (Pretoria, South Africa)

Oral 27
Genetic diversity of rotavirus A strains among children less than 5 years old with acute diarrhea in Mozambique
Nilsa DE Deus (Maputo, Mozambique)

Oral 28
Rotavirus epidemiology in four districts of Angola before vaccine introduction
Claudia ISTRATE (Lisboa, Portugal)

Oral 29
Phylogenetic analysis of human group C rotavirus circulating in Brazil reveals a potential unique NSP4 genetic variant and high similarity with Asian strains
Adriana LUCHS (Sao Paulo, Brazil)

10:00 - 10:30
Coffee break in the exhibition and poster areas

10:30 to 12:00
Session VI: Rotavirus infections and Molecular epidemiology in Europe
Chair by Javier BUESA and Miren ITURRIZA GOMARA

Oral 30
Hospital-acquired rotavirus and norovirus gastroenteritis in Italian children, in 2014-2015
Diletta VALENTINI (Rome, Italy)

Oral 31
Patterns of rotavirus strain circulation across twelve European countries prior to the introduction of routine rotavirus vaccination – 2007/08-2012/13
Daniel HUNGERFORD (Liverpool, United Kingdom)

Oral 32
Evolution of human G4P[8] group a rotavirus strains circulating in Italy in 2013
Giovanni IANIRO (Rome, Italy)

Oral 33
Rotaviruses in hospitalized children with diarrhoea in Slovenia – still the leading cause
Andrej STEYER (Ljubljana, Slovenia)

Oral 34
Surveillance of rotavirus strains in Valencia, Spain, during 12 years (2003-2014)
Jesús RODRIGUEZ-DIAZ (Valencia, Spain)

Oral 35
The Italian Rotanet surveillance program. Rotavirus genotypes among children hospitalized with severe gastroenteritis, 2007-2014
Roberto DELOGU (Rome, Italy)

Oral 36
Intra-genotypic characterization of group A rotavirus strains circulating in Germany (2008-2013)
Andreas MAS MARQUES (Berlin, Germany)

12:00 – 13:00
Lunch and Visit the poster presentations
13:00 - 14:15
Lunch buffet
**Wednesday, 20 May**

7:45 – 9:00  Meeting for delegates of the EuroRotaNet Project

9:00 to 10:10  Session VII: Emerging rotavirus strains
*Chaired by Jelle MATTHIJNSSENS and Hester O’NEILL*

Isidore BONKOUNGOU (Ouagadougou, Burkina Faso)

Oral 38  Emergence and evolution of G12P[8] rotavirus strains in Spain
Javier BUESA (Valencia, Spain)

Oral 39  Introduction of G12 rotaviruses in Sicily in 2012 and sustained circulation until 2014
Giovanni M GIAMMANCO (Palermo, Italy)

Oral 40  Complete genome analysis of a G12P[9] reassortant strain brings to the attribution of novel VP1, VP2, VP3 and NSP2 genotypes
Simona DE GRAZIA (Palermo, Italy)

Oral 41  Transient emergence of G12 rotaviruses in French infants
Alexis de ROUGEMONT (Dijon, France)

10:10 to 10:30  Coffee break in the exhibition and poster areas

10:30 to 12:00  Session VIII: Vaccine effectiveness and epidemiology
*Chaired by Lucia FIORE and Nigel CUNLIFFE*

Oral 42  Large increase of rotavirus diarrhea in the hospital setting associated with emergence of G12 genotype in a highly vaccinated population in Nicaragua
Filemon BUCARDO (Leon, Nicaragua)

Oral 43  Impact of vaccination on rotavirus in the first year after introduction of the Rotarix® vaccine in England
David ALLEN (London, United Kingdom)

Oral 44  Changing pattern of rotavirus genotypes circulating in Australia since vaccine introduction
Carl KIRKWOOD (Parkville - Melbourne, Australia)

Oral 45  Host genetics and rotavirus infections – impact on epidemiology, immunology and vaccine take
Johan NORDGREN (Linköping, Sweden)

Oral 46  Rotavirus genotypes circulating in Greece during the post vaccination era (2008-14)
(Dimitra KOUKOU (Athens, Greece)

Oral 47  G12P[8] species A rotavirus causing gastroenteritis outbreak in different Brazilian regions in 2014: VP7 and VP8* genetic characterization
Marcelle SILVA (Rio de Janeiro, Brazil)

Oral 48  Rotavirus strains circulating in Finland - results from two years of national surveillance, 2013 and 2014, years 5 and 6 after introduction of rotavirus vaccination
Haider AL-HELLO (Tampere, Finland)

12:00 - 13:30  Lunch and Farewell
POSTERS

From Sunday, 17 May (20:00) to Wednesday, 20 May

Poster 01  Characterization of the cell cycle arrest induced by rotavirus 
Catherine EICHWALD (Zurich, Switzerland)

Poster 02  Immune responses and protection in mice induced by parenteral and mucosal delivery of VP6 
subunit rotavirus vaccine 
Suvi LAPPALAINEN (Tampere, Finland)

Poster 03  Screening for antibodies and their cognate epitopes that can block rotavirus-host cell interaction 
in the porcine model 
Nathan MEADE (Nottingham, United Kingdom)

Poster 04  Epidemiological and phylogenetic analysis of avian rotaviruses in Italy 
Chiara BUSI (Brescia, Italy)

Poster 05  Partial genomic analyses of Moroccan caprine rotavirus strains provide evidence for interspecies 
transmission 
Sanaa ALAOUI AMINE (Rabat, Morocco)

Poster 06  Circulation of pig group A and C rotaviruses in Belgian diarrheic suckling pigs and its impact on 
veterinary diagnostical analyses 
Sebastiaan THEUNS (Ghent, Belgium)

Poster 07  Isolation, identification and virological characterization of bovine rotaviruses from dairy calves, 
Morocco 
Imane ENNIMA (Rabat, Morocco)

Poster 08  Monitoring of rotavirus genotypes in indigenous children of Brazilian Midwest in the vaccine era: 
footprints of animal genome 
Adriana LUCHS (Sao Paulo, Brazil)

Poster 09  Molecular characterization of group A rotavirus genotypes in Oman between 2009 and 2013 
Said AL BAQLANI (Muscat, Oman)

Poster 10  Phylogenetic analysis of VP4 and VP7 coding sequences of Mozambican rotavirus strains 
Hester O’NEILL (Bloemfontein, South Africa)

Poster 11  Rotavirus genotypes during pre-vaccine period in Ouagadougou, Burkina Faso 
Nafissatou OUEDRAOGO (Ouagadougou, Burkina Faso)

Poster 12  Surveillance of rotavirus strains in Valencia, Spain, during 12 years (2003-2014) 
Jesús RODRIGUEZ-DIAZ (Valencia, Spain)

Poster 13  The Italian Rotanet surveillance program. Rotavirus genotypes among children hospitalized with 
severe gastroenteritis, 2007-2014 
Roberto DELOGU (Rome, Italy)

Poster 14  Intra-genotypic characterization of group A rotavirus strains circulating in Germany (2008-2013) 
Andreas MAS MARQUES (Berlin, Germany)

in Italian children in 2009 
Giovanni IANIRO (Rome, Italy)

Poster 16  Transient emergence of G12 rotaviruses in French infants 
Alexis de ROUGEMONT (Dijon, France)

Poster 17  One year survey of human rotavirus strains suggests the emergence of genotype G12 in Apulia 
region (South Italy) 
Maria CHIRONNA (Bari, Italy)
Poster 18  Unexpected diffusion of G12P[8] rotavirus strains among young children in a small area of central Italy  
Roberto DELOGU (Rome, Italy)

Poster 19  Rotavirus genotypes circulating in Greece during the post vaccination era (2008-14)  
Dimitra KOUKOU (Athens, Greece)

Poster 20  G12P[8] species A rotavirus causing gastroenteritis outbreak in different Brazilian regions in 2014: VP7 and VP8* genetic characterization  
Marcelle SILVA (Rio de Janeiro, Brazil)

Poster 21  Rotavirus strains circulating in Finland - results from two years of national surveillance, 2013 and 2014, years 5 and 6 after introduction of rotavirus vaccination  
Haider AL-HELLO (Tampere, Finland)

Poster 22  Clinical and molecular descriptions of rotavirus in Morocco 2 years after Rotarix® introduction  
Doblali TAOUFIK (Rabat, Morocco)

Poster 23  Predominance of G12 rotavirus strains in Barcelona, Venezuela, following the introduction of the vaccine  
Esmeralda VIZZI (Caracas, Venezuela)

Poster 24  Evaluation of a Taqman Array Card test for group a rotavirus detection and genotyping in Brazilian stool samples  
Irene ARAUJO (Rio de Janeiro, Brazil)

Poster 25  Diagnostic accuracy of seven commercial assays for the rapid detection of group A rotavirus antigens  
Jerome KAPLON (Dijon, France)

Poster 26  Retrospective assessment of Vikia® Rota-Adeno and premier Rotaclone® tests compared to reverse transcription polymerase chain reaction for detection of group A rotavirus  
Adamou LAGARE (Niamey, Niger)

Poster 27  False-positive rotavirus results are not completely avoided by pre-RT-qPCR treatments with propidium monoazide or RNase  
Leena MAUNULA (Helsinki, Finland)

Poster 28  Towards a validation of a serum neutralization test to control human rotavirus vaccine efficacies in Morocco  
Hassane BOULAHYAOU (Rabat, Morocco)
BOOK OF ABSTRACTS

Keynote conference

Immune responses and protective mechanisms to rotavirus infection
GREENBERG Harry
Stanford University, Palo Alto, United States

Isolation and characterization of human monoclonal antibodies with heterotypic neutralization specificity

Keywords: Heterotypic Immunity, Monoclonal Antibodies

INTRODUCTION:
Single natural RV infections and monotypic RV vaccinations induce heterotypic immunity against subsequent severe diarrheal disease. This heterotypic immunity is critical to the success of the Rotarix™ vaccine, which contains only a single strain of attenuated human RV. The goal of this study was to elucidate possible mechanisms responsible for heterotypic humoral immunity to RVs in humans by analyzing the specificity of immunoglobulin genes cloned from RV-specific intestinal B cells.

METHODS:
Intestinal RV specific B cells were isolated from intestinal resections using Cy5-conjugated CDC 9 strain (G1, P[8]) RV TLPs. Intestinal IgA+ antibody secreting cells which bound the Cy5-conjugated RV were single cell sorted and subjected to paired heavy and light chain antibody gene sequencing. Rooted evolutionary trees representing RV-specific antibody repertoires were generated from each subject by clustering these sequences. Subsequently, selected mAbs were synthesized, expressed, and characterized for their binding specificity and neutralizing activity against homotypic and heterotypic RV strains in vitro and in vivo.

RESULTS:
We observed that the most of the first group of human mAbs isolated were directed against VP4 (23 total) with many fewer against VP7 (2 total) or VP6 (1 total). 19 of the 23 VP4 mAbs were directed at the VP8* segment of VP4 and none of these neutralized RVs. Four VP4 mAbs displayed RV neutralizing activity and two of these neutralized RVs with multiple P and G serotypes. None of the 4 neutralizing mAbs bound to VP8*.

CONCLUSION:
These results suggest that humans can circumvent the serotypic diversity of circulating RV strains by expressing individual VP4 (or potentially VP7) epitope-specific Ig molecules that mediate heterotypic as well as homotypic neutralization. Characterization of the precise targets of these recombinant mAbs at the protein, serotypic and structural levels and determination of the extent to which they arise following primary RV infection will provide the basis for designing more effective future vaccines.
Session I. Structure-function, replication and assembly

Oral 01 - A conserved translation regulatory motif (TRM) in the 5’UTRs of rotavirus genome segments
DRIKIC Marija, DE LORENZO Giuditta, EICHWALD Catherine, BURRONE Oscar R., ARNOLDI Francesca
International centre for genetic engineering and biotechnology, Trieste, Italy.
Keywords: Regulatory motif, Viral translation, Genome segments
Rotavirus genome segments are composed of a single open reading frame (ORF) flanked by untranslated regions (UTRs). The function of UTRs is still not well elucidated but they are believed to play a role in genome replication, genome packaging, and in regulation of gene expression; the 5’-terminal consensus 5’-GGC(A/U)7-3’ and the 3’-terminal consensus 5’-U(G/U)3(A/G)CC-3’ sequences are common to all eleven segments. In the present work we investigate the role of UTRs. We tested in MA104 cells constructs containing the full-length viral segment 11 (5’UTR, NSP5 ORF, 3’UTR) and constructs lacking one or both the UTRs. A strong inhibitory effect due to the 5’UTR primary sequence was observed. The decreased protein expression was not dependent on the ORF or the 3’UTR sequence. Quantitative RT-PCR showed that transcription was not impaired leading to the conclusion that the reduced expression was due to translation. Analysis of several mutants of the 21-nucleotide long 5’UTR of gs 11 defined a translation regulatory motif (TRM) represented by its primary sequence rather than its secondary structure. The 5’UTR sequence of genome segment 8 (NSP2 in SA11) shared this inhibitory activity whilst the 5’UTR of genome segment 2 (VP2) did not. The alignment of the 5’ termini of the three segments revealed that an 11-nucleotide long U-A rich motif was conserved with a single difference (A5 instead of U5) in segment 2. Mutation of A into U in position 5 in genome segment 2 conferred inhibitory activity to the relative 5’UTR, while U to A mutation abrogated that of the 5’UTR of genome segment 11. We mapped the TRM and defined it as the 5’ terminal hexanucleotide 5’-GGY(U/A)UU-3’. The 5’ terminal position is essential to maintain inhibitory capacity. We tested the 5’UTRs of all genome segments cloned upstream of the EGFP ORF. As expected, EGFP expression was strongly decreased by 5’UTRs containing a TRM sequence (gs 3, 5, 6, 7, 9, 10; SA11 strain) whilst it was not with those of gs 1 and 4 that do not contain a functional TRM (both with A in position 5). Rotavirus infection partially reverted the TRM-mediated down-regulation. Our data suggest a function of 5’UTRs in modulation of viral protein translation. Interestingly, the TRM sequence and its non-functional version are highly conserved among the same genome segments of different rotavirus strains of group A. Furthermore, the poor translation efficiency caused by the TRM motif could explain the failure of the numerous attempt to obtain helper virus-free reverse genetics systems.

Oral 02 - Early events in viroplasms formation require association with the molecular motor dynein
EICHWALD Catherine, GLUCK Selene, SCHRANER Elisabeth M., MATTHIAS Patrick, ACKERMANN Mathias
Institute of Virology, University of Zurich, Zurich, Switzerland
Keywords: Dynein, viroplasm, molecular motor
INTRODUCTION:
We have previously demonstrated that viroplasms are able to coalesce and migrate towards the perinuclear area through a mechanism mediated by stabilized microtubule (MT)-network and by molecular motors. One of these molecular motors was the kinesin Eg5, which has a role in the formation and in the maintenance of the viroplasmic structure, as well as its migration towards the perinuclear area. We hypothesized that additional molecular motors might be involved in formation of viroplasms and their dynamics. Dynein may be such a candidate because it belongs to the AAA+ superfamily, transporting cargoes towards the MT minus end, which lies in the MTOC near the nucleus in non-dividing cells. Dynein powers the transport of membrane-bound vesicles and tubules as endosomes, lysosomes, phagosomes, melanosomes, peroxisomes, lipid droplets, mitochondria and ER vesicles destined to Golgi.
OBJECTIVES:
Characterize the role of dynein in the formation and dynamics of rotavirus viroplasms and to establish the presence of direct interaction of rotavirus proteins with this molecular motor complex.

METHODS:
Diverse cell biology methodologies were employed in this study, including confocal cell imaging, viroplasms quantification, electron microscopy, binding assays for protein interaction in the presence of specific drug inhibitors for host components.

RESULTS:
When the dynein subunit dynactin (p50-EGFP) was over-expressed in RV infected cells, morphologically defective viroplasms were observed. Additionally, we detected very few viroplasms distributed in cell periphery after using the specific dynein inhibitor ciliobrevin D at early times post-infection (1hpi) and then analyzing the viroplasms dynamics at later time post-infection (6hpi). This was also concomitant with a reduction in viral progeny. Interestingly, viral protein content was reduced in VP2, NSP4 or VP6 but not in NSP5. It is well known that NSP5 gets hyper-phosphorylated, O-NAc-glycosylated and also covalently sumoylated. The sumoylation, even though not well defined for NSP5, has been attributed to support protein interactions. When inhibiting dynein, NSP5 sumoylation was abolished in favor of NSP5 ubiquitination. Interestingly, when using ciliobrevin D in conditions where MTs are hyper-acetylated, as when using HDAC6/- cells or treated with tubacin, we detected an increase in the degradation of rotaviral proteins. Similar observations were also obtained in a simplified model using viroplasm-like structures induced by co-expression of NSP5 with VP2.

CONCLUSIONS:
Early events in the formation of viroplasms require the association of early expressed rotavirus proteins with dynein in order to get sumoylated. At a first glance, our data suggest that NSP5 is the primary rotavirus protein that gets sumoylated after its association with dynein. A pre-requirement for the stabilization of MTs seems to be in fine tune with this proposed pathway.

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Oral 03  - Generation of an avian/mammalian rotavirus reassortant using a helpervirus-dependent reverse genetics system

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**Keywords:** avian/mammalian reassortant, reverse genetics system, host tropism

Rotavirus A (RVA) strains have been detected in a wide range of animal species and humans. Genome sequence analyses identified several RVA reassortants, which are supposed to be a result of zoonotic transmission between mammalian species and humans. Although avian RVAs are genetically only distantly related to mammalian and human RVAs, their zoonotic potential and their ability to exchange genome segments with other RVAs remains unknown.

To investigate the host tropism of avian and mammalian rotaviruses, primary chicken embryo fibroblasts (CEF) and the monkey kidney cell line MA104 were infected with the human RVA strain Wa and the chicken RVA strain Ch02V0002G3. Both viruses replicated in these cell types, however, only with low efficiency in CEF. In MA104 cells, the chicken RVA strain replicated less efficient than the human Wa strain. Preliminary co-infection trials with both viruses did not result in the isolation of reassortant viruses, presumably due to the different replication kinetics in these cells. To determine if avian RVA segments can be packaged by their mammalian counterparts, the VP4-encoding genome segment of the avian RVA strain Ch02V0002G3 was cloned under the control of a T7 RNA polymerase promotor and HDV ribozyme. The clone was transfected into BSR-T7/5 cells, which stably express the T7 RNA polymerase. The transfected cells were infected with the monkey RVA strain SA11-tsA, which contains a temperature-sensitive mutation in its VP4 gene. After selection of generated viruses at high temperatures on MA104 cells, an SA11 reassortant containing the VP4 gene of strain Ch02V0002G3 could be isolated. The results show that avian and mammalian RVAs are capable of replication in respective cell cultures. In addition, viable reassortants can be generated from both viruses, thus broaden the spectrum of genetic variability of RVA in mammalian and avian hosts.
Oral 04 (short) & Poster 01 - Characterization of the cell cycle arrest induced by rotavirus

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*Keywords: cell cycle, kinesin, viroplasm*

**INTRODUCTION:**
The rotavirus (RV) replication machinery requires a stabilized cytosolic microtubule-network and also the activity of kinesin Eg5. At the onset of mitosis and interphase the MT-network gets depolymerized, allowing the nucleation of short microtubules at the centrosomes, following the spindle assembly. We hypothesize that such rearrangements are detrimental for RV replication, making interference with MT-break-down essential, which in turn arrests the cell cycle prior to mitosis.

**OBJECTIVES:**
Demonstration of RV-induced cell cycle arrest and elucidation of the mechanism, by which RV achieves the MT-stabilization that lead to the cell cycle arrest.

**METHODS:**
We used synchronized RV-permissive cell lines, at the onset of the S-phase and monitored the cell cycle progression after infection with RV by staining with propidium iodide, followed by flow cytometry.

**RESULTS:**
Compared to non-infected cells, different RV strains, like the simians SA11 and RRV as well as the porcine OSU, were able to arrest infected cells in the S/G2 phase. In addition, we found that cell lines CV-1, Caco-2 and MDCK were also arrested in S/G2 phase upon RV infection. Interestingly, when RV was inactivated with UV-psoralen, which allows internalization of transcriptionally negative virions, the cell cycle arrest could not be observed. Additionally, we found that Eg5, which normally localizes near centrosomes, was re-located in the cytosol surrounding the RV-viroplasms in infected cells. Finally, we show a series of experiments aimed at elucidating the responsible viral component involved in the cell cycle arrest and in the re-distribution of the molecular motor surrounding the viroplasms.

**CONCLUSIONS:**
Our results highly suggest that RV uses a common pathway for the arrest of the host cell cycle that is RV strain and cell-line independent. RV impedes MT breakdown by arresting the cell cycle prior to mitosis (S/G2 phase), through a mechanism that is common between different RV strains and permissive cell lines. In addition, this mechanism requires viral transcription, suggesting a role of early expressed RV proteins. The re-localization of Eg5 upon RV infection, suggests a subversion of this host component that results in MT-stabilization in the interphase.
Session II. Virus-cell and virus-gut interactions

Oral 05 - The cholinergic anti-inflammatory pathway attenuates inflammation during rotavirus infection

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**Keywords:** Rotavirus, Inflammation, Vagus nerve

**INTRODUCTION:**
Rotavirus (RV) causes acute gastroenteritis in young children characterized by severe diarrhea and vomiting. Although the infection results in significant intestinal pathology, the inflammatory response is remarkably limited.

**OBJECTIVE:**
We tested the novel hypothesis that RV infection stimulates the cholinergic anti-inflammatory pathway via the vagus nerve to suppress gut inflammation.

**METHODS:**
The role of the vagus nerve and the α7 nicotinic acetylcholine receptor (α7 nAChR) in the inflammatory response to RV infection were explored in α7 nAChR gene-deficient mice, vagotomized mice and wild type mice treated with the α7 nAChR antagonist mecamylamine. Following oral inoculation with murine RV, the levels of TNF-α, IL-1β and IL-6 were measured by ELISA, in serum, spleen, duodenum, ileum and jejunum at 48 hours post infection. To investigate if stimulation of the α7 nAChR pathway could attenuate the release of pro-inflammatory cytokines in vitro, mouse peritoneal macrophages and human blood monocyte-derived macrophages, were treated with nicotine before stimulation with NSP4, the enterotoxin encoded by RV. To determine if modulation of the inflammatory response affects virus shedding, α7 nAChR was blocked with mecamylamine in infected mice and virus quantified in faeces.

**RESULTS:**
Stimulation of the vagus nerve and α7 nAChR-mediated signaling attenuated the pro-inflammatory response during RV infection. Similarly, nicotine attenuated the release of TNF-α and IL-6 from macrophages stimulated by NSP4 in vitro. Moreover, blockade of α7 nAChR attenuated virus shedding from infected mice.

**CONCLUSION:**
The cholinergic anti-inflammatory pathway participate in the attenuation of inflammatory response during rotavirus infection.

Oral 06 - Intracellularly expressed rotavirus NSP4 stimulates release of serotonin (5-HT) from human enterochromaffin cells

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**Keywords:** NSP4, Serotonin (5-HT), Enterochromaffin cells

**INTRODUCTION:**
Rotavirus (RV) is associated with diarrhoea and vomiting, but the mechanisms behind these symptoms remain unresolved. While RV have been shown to infect and stimulate secretion of serotonin (5-hydroxytryptamine; 5-HT) from human enterochromaffin (EC) cells and to infect
EC cells in the small intestine of mice, the intracellular viral protein responsible for this novel property remains to be identified.

OBJECTIVE:
The aim of this study was to investigate the role of NSP4 in 5-HT release from EC cells during RV infection.

MATERIAL AND METHODS:
To address this issue, human EC cells were transfected with small interfering RNA (siRNA) targeting major structural (VP4, VP6 and VP7) and the non-structural (NSP4) viral proteins (VP) followed by infection with Rhesus rotavirus (RRV). Immunohistochemistry together with Western Blot analysis was used to evaluate the transfection efficiency and 5-HT ELISA was performed to analyse 5-HT release. BAPTA/AM was used to analyse intracellular calcium perturbation. Real-time quantitative PCR was performed on small intestine segments from RV-infected and non-infected mice and analysed for 5-HT transporter (SERT) and tryptophan hydroxylase 1 (TPH1) mRNA expression.

RESULTS:
siRNA specific to NSP4 (siRNANSP4) significantly (p<0.05) attenuated secretion of 5-HT compared to non-targeting (NT) siRNA, siRNAVP4, siRNAVP6 and siRNAVP7. Intracellular calcium perturbation with BABTA/AM revealed that NSP4-stimulated secretion of 5-HT from EC cells was calcium-dependent. Furthermore RV infection down-regulated the 5-HT transporter (SERT) mRNA in ileum but not tryptophan hydroxylase 1 (TPH1) mRNA. The unaffected TPH1 mRNA expression in the intestinal segments suggests that release of 5-HT primarily occurs from pre-made 5-HT rather than from newly synthesised 5-HT mRNA. Moreover, down-regulation of SERT mRNA in ileum presumably resulted in reduced re-uptake of 5-HT to EC cells and thus increased extracellular 5-HT in the small intestine.

CONCLUSION:
In this study, we have shown that intracellularly expressed NSP4 during RV infection significantly stimulate EC cells to increase secretion of extracellular 5-HT and that 5-HT release and Ca2+ levels are two connected cell processes during RV infection.

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Oral 07 - Rotavirus infection induces alternative splicing of the stress-regulated transcription factor XBP1

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Keywords : Stress, UPR, NSP3

The Unfolded Protein Response is a final common pathway in the response of a broad variety of stresses disturbing the Endoplasmic Reticulum (ER), as calcium deregulation or accumulation of unfolded proteins. There are three ER-stress sensors: ATF6, PERK and IRE1. ATF6 is a transcription factor activated by proteolysis and PERK is one of the eIF2a kinases. Metazoan IRE1 is an ER transmembrane anchored kinase and sequence-specific endonuclease. Once activated, IRE1 splices out 26 nucleotides in exon 4 of the XBP1-u (unspliced) mRNA present in the cell cytoplasm, leading to the generation of XBP1-s (spliced) mRNA. XBP1-u encodes an inactive and unstable form of the transcription factor Xbp1 (Xbp-u) whereas XBP1-s encodes the active form (Xbp1-s) of the transcription factor Xbp1. Xbp1-s protein is able to translocate into the cell nucleus and to activate (alone or in combination with ATF6) the transcription of its target genes. These target genes encode ER-resident molecular chaperones like GRP94, GRP78, calreticulin, that associates sequentially with various polypeptides in the ER and assist in their folding allowing cell to recover from stress.

Rotavirus infection induces the Unfolded Protein Response (UPR). In the course of studying stress induced by rotavirus infection, we discovered a new XBP1 mRNA. This mRNA (XBP1-es) is polyadenylated and corresponds to a canonical nuclear splicing of XBP1 pre-RNA, that skips exon 4. XBP1-es encodes an Xbp1 protein (Xbp1-es) 40 amino acids shorter than Xbp1-s, but that contains complete DNA-binding and activating domains. Indeed, Xbp1-es does activate the expression of a luciferase reporter driven by the GRP94, Bip or XBP1 promoters to the same extent than Xbp1-s. XBP1-es was observed nor with cells treated with chemicals commonly used to induce ER-stress and UPR (tunicamycin or thapsigargin) nor with cells treated with translation inhibitors puromycin or cycloheximine. The XBP1-es was detected upon infection with several cell culture-adapted rotavirus strains ("ES+ strains"), but not with
Oral 08 - Selective activation of the unfolded protein response by viral complexes is essential for efficient rotavirus production

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**Keywords: Unfolded Protein Response, viral complexes, selective activation**

Rotavirus infection is accompanied by endoplasmic reticulum (ER) stress, which then triggers the Unfolded Protein Response (UPR). However, the underlying activation mechanisms and the biological role of this adaptive host response in virus infection remain unclear. Here, a set of rotavirus assembly intermediates that form in cytosolic viroplasms and then enter the ER, were purified and characterized using proteomics. We demonstrated that these viral complexes, as entities, recruit chaperones, including the ER resident BiP/GRP78, providing a molecular link between rotavirus budding within the ER and activation of the UPR. Moreover, activation of PERK and IRE1 pathways is required for virus production, as demonstrated by the use of drugs interfering specifically with UPR. Interestingly, ATF6 pathway was attenuated by sequestration within viroplasms and thus not functionally involved in virus production. Hence, our results define the molecular mechanisms of rotavirus-induced UPR initiation and provide evidence that rotavirus selectively hijacks UPR signals to achieve its morphogenesis. This raises the possibility to use pharmacological inhibitors of UPR to fight against rotavirus infections.

Oral 09 - Rotavirus morphogenesis doesn't require autophagy but blocks both its initiation and fusion steps in intestinal epithelial Caco-2 cells

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**Keywords: Autophagy, Intestinal cells, autophagy-independent LC3 lipidation**

Viruses have evolved a large array of strategies to cope with the autophagic process. Whether autophagy may be involved in the morphogenesis of rotavirus was investigated in the present work. We used Caco-2 cells, an intestinal epithelial cell line that mimics the natural target of rotavirus. We first observed that, independent of rotavirus infection, the autophagic status was strongly dependent on cell differentiation, i.e. differentiated Caco-2 cells express higher levels of major ATG proteins involved in autophagy initiation and showed a decreased autophagic flux, leading to autophagosomes accumulation. Rotavirus infection (i) apparently did not modify the steady state of basal autophagy in both undifferentiated and differentiated cells, (ii) blocked the initiation and fusion steps in both conditions (iii) specifically increased LC3 lipidation in undifferentiated cells and (iv) did not promote interactions between rotavirus and autophagosomes. Conversely, manipulation of autophagy with chloroquine and/or rapamycin did not affect virus production, indicating that the autophagic process was disconnected from virus morphogenesis. Using siRNAs against BECN1 or ATG13 we demonstrated that rotavirus-induced LC3 lipidation, observed in undifferentiated cells, was independent on canonical autophagy. Our results indicated that neither autophagy, nor LC3 lipidation nor even LC3 expression played a role in rotavirus production in Caco-2 cells.
Oral 10 - Role of histo-blood group antigens in human RVA infection


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Keywords: Attachment, glycan, histo-blood group

INTRODUCTION:
Human strains of rotavirus A (RVAs) recognize fucosylated glycans of the histo-blood group family (HBGAs) as well as gangliosides through the VP8* protein domain of their capsid. The relative contribution of each of these types of ligands on infection remains unclear. Nevertheless, it has been shown that interaction with gangliosides is essential for cell entry and that the lack of fucosylated ligands due to HBGAs genetic polymorphism is associated with resistance to severe RVA gastroenteritis.

OBJECTIVES:
Our goals are to delineate the relative contribution of HBGAs and gangliosides in the infection process and to explore the consequences of HBGAs polymorphisms on the virus transmission and efficacy of the available live vaccines.

METHODS:
binding to glycans of recombinant VP8*-GST fusion proteins from several French clinical strains and from the vaccine strains were analyzed using glycan microarrays, as well as saliva mucins from groups of individuals representing the major combined ABO, FUT2 and FUT3 HGBA subgroups. MA104 and HT29 cells were used to study attachment and infection by the HAL 1166, Wa and SA11 strains. Expression of HBGAs and gangliosides on these cells was determined by flow cytometry using a set of specific reagents and expression of these glycans was modulated by specific inhibitors of synthesis.

RESULTS:
We observed that the fucosylated glycans could serve as specific attachment factors of either the Wa or the HAL 1166 strains upon preincubation at 4°C, but that their presence was completely dispensable for infection at 37°C, unlike that of gangliosides. In addition, the VP8* from several recent (2011-2012) French clinical strains belonging to the [P8] genotype showed binding to difucosylated structures as well as to saliva mucins from individuals who express both FUT2 and FUT3 functional fucosyltransferases (secretors/Lewis positive phenotype), with little effect of the ABO phenotype. The lack of either enzyme or of both enzymes led to an absence of binding to the saliva samples, as previously reported for older strains of the same genotype. Interestingly, VP8* from the vaccine strains showed the same binding profile to either glycan microarrays or to saliva samples, demonstrating a remarkable conservation within the [P8] genotype.

CONCLUSIONS:
The concordance between the glycan specificity of [P8] strains VP8* and the previously reported HBGAb-dependant susceptibility to severe RVA gastroenteritis indicates that HBGAbinding of human RVA strains is essential to the infection process. Yet, our results suggest that HBGAbinding of human RVA strains corresponds to an early event since it is not required at the level of cellular infection, at least of the poorly differentiated cells that we used. The use of more sophisticated cellular models will be necessary to clarify this point. In addition, the fact that HBGAb recognition is conserved between recent [P8] strains and the vaccine strains, suggest that HBGAb polymorphism may contribute to explain the low efficacy of the vaccines in some geographical areas where the frequency of FUT2 negative or FUT3 negative individuals is high.
Oral 11 - Relationship between rotavirus infection and blood group antigens

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Keywords: Rotavirus, HBGAs

INTRODUCTION:

Enteric viruses are a major cause of gastroenteritis in Tunisia. Group A rotavirus is the leading cause of AGE in children under 5 years old, and is estimated to cause around 453,000 deaths yearly, mostly in developing countries like in North Africa.

MATERIALS AND METHODS:

One hundred fourteen matched saliva, blood and stool samples were collected from children (N=114) below 6 years of age suffering from acute gastroenteritis and who visited a pediatrician at the hospital of Monastir during the winter season 2011-2012. The stool suspension was first screened for the presence of group A rotavirus antigen using an enzyme-linked immunosorbent assay (ELISA). The RV-positive specimens were then genotyped by PCR. The saliva samples were typed for the presence of HBGAs (human blood group antigens: A, B and H antigens and Lewis antigens). The blood samples (N=102) were used to determine secretor genotype by PCR.

RESULTS:

Rotaviruses (RVs) were responsible for 28.1% of the gastroenteritis and G9P[8] was the predominant RV (37.5%). For patients who were phenotyped (N=114) and genotyped (N=102) for human blood group antigens (HBGAs), the secretor and non-secretor phenotype represented 79 and 21%, respectively. Rotaviruses were detected among secretor (N=28) and non-secretor (N=4) individuals. Additionally, RVs were detected in non-secretor individuals and for all blood types among the secretors. No significant association was found between ABO antigens or the secretor status and RV infection. Nevertheless, we observed that RV infection always occurred in Lewis-positive patients (P=0.017, exact logistic regression).

CONCLUSION:

Rotavirus infections have been observed in individuals belonging to ABO groups and non-secretor individuals. Besides integrins, our study suggests that Lewis antigen might be natural ligand for human group A RVs. Large scale studies will be necessary to exactly determine which RV genotypes could infect secretor and non-secretor individuals among population with different genetic background.

Oral 12 - Human rotavirus usage of glycans as cellular receptors

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Keywords: human rotavirus receptors, histo-blood group antigens, sialic acid

INTRODUCTION:

Previous work implicated histo-blood group antigens (HBGA) as cellular receptors for human rotaviruses (hRV), typically for rarer hRV serotypes. This led to several studies of the relation of blood type and secretor status with childhood hRV susceptibility, with apparently somewhat inconsistent findings. Internal (branched) N-acetylgalactosaminic acid (Sia) on ganglioside GM1 has been shown to act as a cellular receptor for a representative common hRV, strain Wa. Bacterial sialidases in the human gut may facilitate hRV infection, by removing main chain (terminal) Sia from epithelial cells. The VP8* subunit of virus spike protein VP4 binds these glycans.

OBJECTIVES:

To conduct structure-function analyses of HBGA and Sia for (i) binding by VP8* of common and rarer hRV serotypes, and (ii) usage as receptors by these hRV on epithelial cells.

METHODS:
Wa [P(8)], RV-3 (P(6)), ST-3 (P(4)), DS-1, K8 (P(9)) and HAL1166 (P(14)) hRV, their expressed VP8* and MA104 cells were used. Several NMR methods, homology modelling and MD simulation were applied in studies of VP8*-glycan interactions. VP8*-cell binding and its inhibition by glycans was determined by flow cytometry. Glycan effects on hRV infectivity were assayed by indirect immunofluorescence.

RESULTS:
NMR studies showed interaction of DS-1 and RV-3 VP8* with type A HBGA, involving the fucose moiety. K8 and HAL1166 VP8* also bound type A HBGA without engaging the fucose. K8 VP8* binding affinity was less than HAL1166 VP8*. Wa VP8* did not bind type A HBGA. No VP8* bound Lewis b or H type-1 antigens. VP8*-cell binding and/or infectivity studies reflected these findings, with type A HBGA inhibiting DS-1, RV-3, ST-3, K8 and HAL1166 but not Wa, and Lewis b and H type-1 antigens having no effect. The VP8* of Wa and RV-3 bound the internal Sia on ganglioside GM1. VP8* binding to aceramido-GM1 was detected by STD NMR. VP8* binding to GM1 occurred at the cell surface, and facilitated host cell invasion by hRV. Supplementation of cellular GM1 levels with exogenous GM1 specifically increased the infectivity of Wa and RV-3. Removal of terminal Sia on cells by treatment with a bacterial sialidase also increases GM1 availability and hRV infectivity.

CONCLUSIONS:
Common hRV types, P(4) and P(6), can use type A HBGA as cellular receptors, with VP8* binding via the HBGA-defining fucose residue. Rarer hRV of P(9) and P(14) and their VP8* also bind type A HBGA, but the HBGA-defining fucose moiety does not interact with their VP8* so this interaction may not be entirely specific. Representative P(6) hRV strain RV-3 also utilises the internal Sia on cell surface GM1 glycan for infection, via VP8* binding. Thus, VP8* on RV-3 can bind both internal Sia and type A HBGA to facilitate cell entry. As the P(14) VP8* binding site for type A HBGA overlaps the binding site for main chain terminal Sia identified for animal rotaviruses, it is proposed that RV-3 binds both type A HBGA and internal Sia via a similar site, potentially revealing a plasticity in VP8* for receptor binding that is unusual for viruses.

Session III: Immune responses - immunopathological diseases

Oral 13 - Rotavirus NSP1 down-regulates levels of multiple interferon receptors on intestinal epithelial cells

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Keywords: NSP1, stat1, interferon

INTRODUCTION:
Rotavirus (RV) replicates robustly and efficiently inhibits interferon (IFN) induction in single infected and bystander villous intestinal epithelial cells (IECs) in vivo. Paradoxically, RV also induces type I IFN expression in the intestinal hematopoietic compartment in a relatively replication-independent manner. This suggests that RV replication and spread in IECs occur despite exogenous stimulation of STAT1-mediated IFN signaling.

OBJECTIVES:
Recently, we showed that RV can inhibit STAT1 Y701 activation in infected as well as bystander IECs that are exposed to exogenous IFN, and that direct STAT1 suppression can be recapitulated by expression of the RV NSP1 protein. Our objective here was to dissect the underlying mechanisms behind RV mediated inhibition of IFN signaling. Here we report that RV mediates a potent decrease in the expression of interferon receptors (IFNRs).

METHODS & RESULTS:
Infection of IECs with 2 RV strains that inhibit IFN induction by distinct strategies targeting either NFKB or IRF3 resulted in effective downregulation of IFNR levels. The effects of different RV strains on the normal turnover of IFNRs at various times during infection were compared and correlated to regulation of STAT activation at the single cell level. Decreases in IFNR were mediated by transient expression of the RV NSP1 protein alone, and critical regions
on NSP1 that mediated the receptor decrease were identified. The effect of RV infection on levels of IFNR was examined in suckling mice infected with murine RV, and the data obtained indicate that significant decreases in IFNR also occur during RV infection in vivo.

CONCLUSIONS:
These results enrich existing models of RV inhibition of host IFN responses, and demonstrate that during infection, RVs deplete IFNRs critical to STAT1 signaling downstream of types I, II, and III IFN-mediated antiviral signaling. Our findings also extend and explain previously observed remote regulation of STAT1 and suppression of the host IFN response in vivo by rotavirus, and reveal the basis for NSP1’s effects on both IFN induction and signaling.

Oral 14 - Acceleration of type 1 diabetes development by rotavirus is associated with type 1 interferon-dependent responses in regional lymph nodes
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Keywords: immune responses to rotavirus in mice, mouse models of type 1 diabetes, rotavirus acceleration of type 1 diabetes.

INTRODUCTION:
Infection by Rhesus monkey rotavirus RRV accelerates type 1 diabetes onset in non-obese diabetic (NOD) mice with pre-existing islet autoimmunity. Diabetes acceleration is associated with presence of infectious RRV in dendritic cells (DC) within the mesenteric (MLN) and pancreatic (PLN) lymph nodes but not islets. Furthermore, RRV infection induces DC maturation, increases the ability of B cells to present antigen to autoreactive T cells and elevates proinflammatory cytokine expression by T cells in these lymph nodes. NOD mouse-derived plasmacytoid DC (pDC) stimulated with RRV ex vivo can induce bystander lymphocyte activation, including the activation of autoreactive T cells. This activation depends on signalling through the type I interferon receptor (IFNAR). Additionally, NOD cells show heightened DC and B cell activation following RRV stimulation compared to cells from non-diabetes prone C57BL/6 mice.

OBJECTIVES:
To determine if (i) RRV-infected mice show evidence of bystander lymphocyte activation in sites relevant to diabetes, (ii) this activation is heightened in diabetes-prone mice, and (iii) it depends on type I interferon signalling.

METHODS:
NOD mice and NOD.IFNAR1/- mice (unable to signal through the IFNAR) were infected orally with RRV at 12 weeks of age. Virus excretion in stools and titres in lymph nodes were determined by titration in MA104 cells and ELISA. Organs collected at day 3 post infection were analysed for pDC activation by flow cytometry and/or Mx1 and Ifit1 by qPCR.

RESULTS:
RRV infection of NOD mice induced the activation of pDC in the MLN and PLN, but not spleen. Furthermore, robust upregulation of type I interferon-dependent genes, Mx1 and Ifit1, was observed in the MLN and PLN but not spleen or islets. NOD.IFNAR1/- mice showed slightly elevated RRV titres in lymph nodes over NOD mice. Use of NOD.IFNAR1/- mice provided evidence for the importance of signalling through the IFNAR for RRV-induced pDC activation, Mx1 upregulation and lymphocyte activation. Furthermore, pDC activation and Mx1 upregulation following RRV infection of non-diabetes prone C57BL/6 mice were limited to the MLN and reduced in level compared with NOD mice.

CONCLUSIONS:
These findings demonstrate that RRV infection induces pDC activation and type I interferon-dependent responses in the MLN and PLN of NOD mice, and these responses are heightened compared to those in C57BL/6 mice. RRV-induced pDC activation, Mx1 upregulation and lymphocyte activation appear to depend on signalling through IFNAR. These responses are known to be critical for type 1 diabetes development in NOD mice and are linked to T1D onset in humans. These data provide further support for our proposed mechanism in which rotavirus activation of pDC via TLR7 in PLN (where islet autoantigens also accumulate) leads
to type 1 IFN production and bystander activation of pre-existing islet-autoreactive lymphocytes. They provide the foundation for studies to determine whether signalling through the IFNAR is essential for type 1 diabetes acceleration by RRV.

Oral 15 - In vivo and in vitro adjuvant properties of rotavirus VP6 protein

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**Keywords:** VP6, adjuvant effect

**BACKGROUND:**
Our laboratory has developed a combined non-live rotavirus (RV) and norovirus (NoV) vaccine candidate consisting of recombinant polymeric human RV VP6 protein and NoV GII-4 and GI-3 virus-like particles (VLPs). Preclinical immunogenicity studies have shown strong type-specific and cross-reactive RV-specific antibody and T cell responses in mice. Challenge studies with murine RV EDIMwt in mice immunized with rVP6 or double-layered VP2/VP6 VLPs demonstrated significant protection against heterologous RV infection.

**OBJECTIVES:**
This study was aimed to study an effect of rVP6 on NoV-specific immune responses in vivo. Furthermore, activation and maturation of antigen presenting cells (APC) by VP6 in vitro was investigated.

**METHODS:**
Mice were immunized with the suboptimal dose (0.3 µg) of GII-4 or GI-3 VLPs with 10 µg rVP6 and NoV-specific immune responses were measured. Raw 264.7 cell line was incubated in vitro with the rVP6 and different controls, and cell surface molecules expression and cytokine production were tested.

**RESULTS:**
When VP6 was administered to mice in a combination with the suboptimal doses of NoV GII-4 and GI-3 VLPs it exerted an adjuvant effect on NoV-specific antibody responses sparing the NoV VLP antigen dose and broadening the responses. Stimulation of immortalized mouse macrophages (Raw 264.7) with the VP6 in vitro induced a significant increase in antigen presentation molecules (MHC II), co-stimulatory molecules (CD40, CD80 and CD86), and pro-inflammatory cytokines (TNF-alpha and IL-6).

**CONCLUSIONS:**
Our results show that VP6 does not only induce protection against RV infection in vivo but it also induces APC activation and maturation and acts as a potent adjuvant for NoV-specific immune responses. Overall, these findings strongly support the use of RV VP6 protein in a combined RV-NoV vaccine candidate.

Oral 16 - Competitive homing of rotavirus-specific memory B cells towards the gut-associated lymphoid tissues

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**Keywords:** B cells, Homing, rotavirus

We previously showed that intra-rectal immunization with rotavirus (RV) 2/6-virus-like particles (2/6-VLPs) protects adult mice against RV infection. This protection is even higher than that achieved by immunization with 2/6-VLPs administered via the intra-nasal route, because it induces a higher humoral and cellular immune response not only in the colon but also in the small intestine (Agnello et al., J Virol., 2006, 80: 3823-32). RV-specific IgA antibody-secreting cells (ASCs) induced by intra-rectal immunization were found to express high levels of the integrin α4β7, which enables them to bind to the addressin MAdCAM-1 and migrate into the intestinal mucosa, and their number is considerably decreased in the gut of β7-deficient mice immunized by the intra-rectal route. On the contrary, IgA ASCs induced by intra-nasal immunization with 2/6-VLPs express low α4β7 levels and are mostly excluded from the gut. Paradoxically, after intra-nasal immunization, antigen-specific IgA ASCs are significantly increased in the small intestine of β7-deficient mice, demonstrating that
lymphocyte homing is a competitive process and that integrin α4β7 determines not only the intestinal tropism of IgA ASCs elicited in the gut-associated lymphoid tissues (GALTs), but also the intestinal exclusion of lymphocytes primed in other inductive sites (Agnello et al., J Immunol., 2013, 190: 4836-47). Memory B cells (MBCs) play a pivotal role in secondary humoral responses, but their location and homing properties are largely unknown. Here we investigated if also RV-specific MBCs express different homing molecules and localize into different tissues when generated by immunization with 2/6-VLPs administered through different routes. Since there are not specific markers for murine MBCs, those lymphocytes have been identified by FACS analysis, in the blood and lymphoid organs of immunized mice, as isotype-switched B cells (B220+, slgD−, slgG/sIgA+), which lack germinal center-specific markers (GL7+, CD38+) and bind to fluorescent 2/6-VLPs. Whereas only ~40% of antigen-specific MBCs induced by subcutaneous or intra-nasal immunization with 2/6-VLPs express α4β7, virtually all antigen-specific MBCs induced by intra-rectal immunization are α4β7+. Therefore, antigen-specific MBCs induced by intra-rectal immunization preferentially recirculate among the GALTs, such as Peyer’s patches (PPs) and mesenteric lymph nodes (MLNs), which express MadCAM-1, but are instead largely excluded from extra-intestinal LNs. Accordingly, when mice deficient for β7-integrin are immunized with 2/6-VLPs by the intra-rectal route, the number of RV-specific MBCs is reduced in MLNs but increased in peripheral LNs. Likewise, adoptive transfer of antigen-specific MBCs from β7-deficient mice immunized by the intra-rectal route generates fewer antigen-specific ASCs in PPs of recipient mice after oral infection with murine RV, but higher number of ASCs in spleen, in comparison with MBCs from normal mice. On the other hand, antigen-specific MBCs are instead significantly increased in MLNs of β7-deficient mice immunized by the intra-nasal route, suggesting that competition among α4β7+ and α4β7- lymphocytes plays an important role for excluding not only α4β7- ASCs from the intestinal lamina propria but also α4β7- MBCs from the GALTs.

Session IV: Rotaviruses and animal infections

Oral 17 - Phylogenetic evolution of animal rotavirus B

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Keywords: rotavirus B, phylogenetic analysis

INTRODUCTION:
Rotavirus B (RVB) has been first identified in a nationwide outbreak of diarrhea mainly in adults in China, and then has spread in Southeast Asia. Other than human, RVB has been associated with outbreaks or sporadic cases of diarrhea in rats, cattle, pigs and lambs. Moreover, serological surveys of RVB infections showed high antibody prevalence in sera from swine and cattle in Japan, the UK and US. However, molecular characterization of animal RVB strains remains unknown, because it is difficult to be serially propagated them in cell culture. We carried out genetic analysis using multiple animal RVBs detected in Japan over 10 years period, in order to understand ecology and evolution of animal RVBs.

METHODS:
Multiple fecal samples were collected from several different farms in Japan from 2000 to 2011. Viral RNA was extracted from 10% stool suspensions according to manufacturer’s instructions. RT-PCR was performed using sets of specific primers originally designed. The PCR products were sequenced using an automated sequencer. Phylogenetic analysis was performed with MEGA software based on the neighbour-joining method. Phylogenetic trees were constructed by bootstrapping with 1000 replicates. The genetic classification in individual gene was performed on the basis of a cut-off value that estimated according to the definition recommended by the Rotavirus Classification Working Group (RCWG).

RESULTS:
Sequence analyses showed the lengths of nucleotide sequences were variable among human and animal RVBs. Comparison analyses of nucleotide and amino acid sequences within and between RVBs from each host species demonstrated that porcine and bovine RVBs indicated low identities with respect to one another, and also exhibited low identities to other RVBs.
Phylogenetic dendrograms revealed that porcine and bovine RVBs were classified into multiple different clusters, in contrast to other RVBs belonging to monophyletic cluster.

CONCLUSIONS:
Our findings indicate that RVBs, particularly porcine and bovine RVBs have large genetic diversity. The data presented here suggest a hypothesis that the viruses spread through the world with a movement of animals, and have evolved originally and adapted in the land. Further accumulation of genetic and epidemiological data of animal RVBs will be essential to prove the hypothesis.

Oral 18 - Metagenomic identification of group A and B rotaviruses in faces of urban wild rats
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Keywords: wild Norway rats, novel rotavirus A and B strains, metagenomic analysis

Urban wild rats are considered as reservoirs and vectors for several zoonotic pathogens. However, information on their enterically shedded viruses with zoonotic potential is still scare. So far, only a group B rotavirus (strain IDIR) was known to infect laboratory rats, whereas the occurrence of group A rotaviruses (RVAs) in rats was not described. Using a metagenomics approach, the intestinal viral contents from 20 wild Norway rats (Rattus norvegicus) collected in the city of Berlin, Germany, were analyzed by next generation sequencing. By this, sequences with identities to group A and B rotaviruses were identified. Application of a rotavirus B VP1 gene-specific RT-PCR resulted in a 340 bp fragment in 6 of the 20 rat faecal samples, which showed the highest nucleotide sequence identity of 78.6% to the rat rotavirus B strain IDIR. An RVA-specific real-time RT-PCR was positive for 4 of the 20 rat samples. The nearly complete genome sequence of one RVA strain revealed the known genotypes G3, P[3] and N2 for three of the genome segments, whereas the remaining eight genome segments had to be assigned to the novel genotypes I20-R11-C11-M10-A22-T14-E18-H13 due to low sequence identities to known genotypes. The results indicate that rats are hosts of several rotaviruses. Further biological characterization of these rotaviruses is necessary in order to assess their host specificity and risk of transmission to humans.

Oral 19 - Complete genome characterization of recent and ancient Belgian pig group A rotaviruses and assessment of their evolutionary relationship with human rotaviruses
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Keywords: pig rotavirus, evolution, interspecies transmission

INTRODUCTION:
Group A rotaviruses (RVAs) are an important cause of diarrhea in young pigs and children. Remarkably, pig RVAs can harbour a wide diversity of genotypes for their outer capsid proteins VP7 and VP4. However, our knowledge on the composition of the other 9 genes of pig RVAs is scarce, which hampers our full understanding of their evolutionary relationship with human RVAs.

OBJECTIVES:
In the present study, it was aimed to reveal the complete genomes of a selection of Belgian pig RVA strains in order to assess their evolutionary relationship with human Wa-like RVAs. Furthermore, it was aimed to consider the risks for interspecies transmission events of pig RVAs to the human population.

MATERIALS AND METHODS:
The complete genomes of six recent (G2P[27], G3P[6], G4P[7], G5P[7], G9P[13], and G9P[23]) and one historic (G1P[7]) Belgian pig RVA strains were revealed using Sanger sequencing. The 5'- and 3'-terminal sequences were obtained using a modified version of the single-primer amplification method. Sequence analysis was performed using 4Peaks software, and multiple-sequence alignments were executed in MEGA 5.2.2. Substitution models were determined for
each gene segment separately. Maximum likelihood trees were constructed, and pairwise
distances calculated, in order to investigate the relationship between pig and human RVAs.

**RESULTS:**
In contrast to the large diversity of genotypes found for the outer capsid proteins VP4 and
VP7, a relatively conserved genotype constellation (I5-R1-C1-M1-A8-N1-T7-E1-H1) was found
for the other 9 genes in most pig RVA strains. However, the ancient strain possessed an I1
genotype for VP6, whereas T1 genotypes were found for NSP3 genes of 3 strains. One strain
beared the rare E9 genotype for the enterotoxin NSP4. VP1, VP2, VP3, NSP2, NSP4, and NSP5
genomes of porcine RVAs belonged to genotype 1, which is shared with human Wa-like RVAs.
However, for most of these gene segments, pig strains clustered distantly from human Wa-
like RVAs, indicating that viruses from both species have entered different evolutionary paths.
However, VP1, VP2, and NSP3 genes of some archival human strains were moderately related
to pig strains. Phylogenetic and amino acid analysis of the VP6, NSP1, and NSP3 genes and
proteins, as well as amino acid analysis of the antigenic regions of VP7, further confirmed this
evolutionary segregation. Most contemporary human strains that clustered in between pig
RVA strains were suspected interspecies transmission events from pigs to humans, and most
of them beared the P[6] genotype for VP4. Interestingly, 5 of the Belgian strains carried a
gene duplication behind the stop codon of gene segment 11 (NSP5).

**CONCLUSION:**
These results confirm the existence of a clear evolutionary relationship between pig and
human RVA strains, but also indicate that viruses from both host species have entered
different evolutionary paths. It seems that the species barrier is less strict for pig P[6] strains,
but that chances for successful spread in the human population are hampered by the better
adaptation of pig RVAs to pig enterocytes. However, future surveillance of pig and human
RVA strains is warranted.

**Oral 20 - A vaccine strategy against porcine rotavirus using cell lines deficient for innate
antiviral mechanisms**

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**Keywords:** Porcine, Innate, Interferon

**INTRODUCTION:**
Rotavirus is an infectious disease that causes gastroenteritis in many animals including
livestock such as pigs. Outbreaks in pig herds cause poor growth performance, an increase in
morbidity and a higher mortality rate. This leads to a reduction in earnings for farmers,
reduced sustainability and a lower quality of pork worldwide. There is no current porcine
rotavirus vaccine available in the UK. We have devised a vaccine strategy that potentially
produces rotavirus that is dependent on modified cell lines deficient for innate antiviral
mechanisms, in order to replicate efficiently.

**OBJECTIVES:**
Monitor the potential of a vaccine strategy to produce vaccine candidates for the porcine
model, using a number of different techniques.

**METHODS:**
MA104 cell lines deficient for innate antiviral mechanisms, utilising two viral proteins; bovine
viral diarrhoea virus (BVDV) Npro protein and Parainfluenza virus 5 (PIVS) V-protein, have
successfully been produced using lentivirus transduction and characterised using
immunoblotting and immunofluorescence techniques. Serial infection of modified MA104 cell
lines was undertaken using the porcine rotavirus OSU strain. In order to monitor if the
resulting virus has become dependent on the modified cell lines we have carried out plaque
assays alongside RNA profiling. We hope to further assess this method by using deep
sequencing to monitor the mutations that have occurred in the genome.

**RESULTS:**
The modified cell lines we have produced render certain properties of rotavirus that
antagonise the innate immune response redundant during viral replication. This is due to the
degradation of specific proteins involved in the IFN response. There was no discernible
difference in the viral titre of OSU that had been serially passaged through the modified cell
lines when plaque assayed on both the modified and naïve MA104 cell lines. In addition,
there has been little or no alteration in the RNA profile of OSU after 30 serial passages. Further assessment of the passaged virus using deep sequencing will help us determine changes to the viral genome that have occurred using this method.

CONCLUSIONS:
Cell lines utilising BVDV Npro and PiV5 V-protein successfully render certain innate antiviral mechanisms inactive in MA104 cell lines. Initial findings suggest that rotavirus serially passaged through these modified cell lines does not become dependent on them for efficient replication. Further assessment is needed to determine this.

Oral 21 (short) and Poster 04 - Epidemiological and phylogenetic analysis of avian rotaviruses in Italy

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Keywords: Avian, phylogenetic analysis

INTRODUCTION:
Avian rotavirus (AvRV) causes enteritis of variable severity in young birds inducing severe economic losses to poultry industry. RVs are classified into eight groups (A-H) but only groups A, D, F and G were reported in avian species. Monitoring rotavirus distribution in both poultry (chickens and turkeys) and game birds is crucial to uncover strains diversity and to understand AvRV epidemiology in the field. Several studies on RVs occurrence and epidemiology in different avian species were reported worldwide. However, very little is known about the characterization of avian RVs in Italy.

OBJECTIVES:
The aim of this study was to provide information on: 1) distribution of the different RV groups in avian species suffering enteritis in Italy; 2) genetic diversity of RVs in these species; 3) dynamics and timing of RV infection within turkey flocks.

METHODS:
To study the distribution of RV groups, we analysed a total of 117 intestinal contents and/or faecal samples collected during the period 2006-2012 from birds of different species (76 chicken; 21 turkey; 10 pheasant; 5 guinea fowl; 5 partridge) all suffering enteritis and resulted positive for rotavirus by electron microscopy. To study the dynamics of infection, a longitudinal study was performed in 4 turkey flocks. Samples were weekly collected from grounding until 27-42 days old. Extracted viral RNA was subjected to RT-PCR assays with specific primer pairs for NSP4, VP6, VP4, VP7 of RV-A and RV-D groups and VP6 of RV-G and RV-F groups using the OneStep-RT-PCR kit (Qiagen). Nucleotide sequences were obtained using the same primers. Sequence alignment was performed using the CLUSTAL W method and phylogenetic trees were constructed using neighbour-joining method.

RESULTS:
One hundred and seven samples out of 117 (91.4%) were positive for group D AvRVs, 70 (59.8%) for group A, 61 (52.1%) for group F and 31 (26.5%) for group G. Single infections were present in 20 samples (17%) and multiple infections were present in 97 samples (83%) with different patterns. A group of 36 positive samples, representative by year and species, were selected for sequence analysis. Phylogenetic trees for VP4, VP7, NSP4 and VP6 segments were constructed on the basis of 115 complete nucleotide sequences. No correlation between year of isolation or avian species and the different RV-groups were observed in all the analysed segments. In some cases, segments of the same sample clustered in different clades evidencing gene reassortment events. Phylogenetic analysis of samples collected during the longitudinal study from 4 turkey flocks showed that different RV-groups and different strains from the same group were present in the same flock. However, such viral pattern changed along the study period within the farm.

CONCLUSIONS:
This study provides novel data on RVs prevalence in avian species in Italy showing both high presence of RV-A, D, F, G groups and great genetic variability. Longitudinal study in turkey flocks evidenced complex RV groups/strains patterns which change over time. Overall, these findings give the basis for further genomic and epidemiological studies aimed to better understand the characteristics of AvRvs circulating in Italy.
Oral 22 (short) and Poster 05 - Partial genomic analyses of Moroccan caprine rotavirus strains provide evidence for interspecies transmission


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**Keywords:** Caprine Rotavirus, Interspecies transmission, Nomadic lifestyle

The genome of group A rotaviruses (RAV) consists of 11 segments of double-stranded RNA. Of these, seg-4 and seg-9 encoded VP4 and VP7 are the major serotype determining proteins P-serotype and G-serotype respectively. Up to date, 27 G types and 35 P types are found worldwide. A co-circulation of different RVA serotypes may provide an opportunity to RVA strains to simultaneously infect the same animal species and/or humans with the potential for generation of reassortant viruses. The Bedouin livestock farming systems may favor the introduction of new strains from a heterologous host by interspecies transmission given their nomadic lifestyle. This preliminary study was carried out to provide some insights into the circulation of RVA in nomadic goats (19 kids (Chèv 1-19) and 5 ewes (Chèv 24) during a severe outbreak of diarrhea in April 2012 in Bouarfa, Eastern part of Morocco. These animals were raring with camels, sheep and cattle. RVA infection was determined with LSI VetMAX™ Triplex Ruminant Rotavirus & Coronavirus Real-Time PCR kit and the frequency of 40% was obtained. All positive samples were subjected to genotyping. RVA G10P[14] was detected in 56% of kids (Chèv 3, 4, 7, 8, 20) and G6P[14] genotype was found in one ewe (Chèv 21). Three cases (Chèv 1, 2, 22) have at least one G and/or P RVA untypable strains representing a portion of 33%. We carried out length sequencing of the genes encoding VP7 and VP 4 of two (Chèv 8 and Chèv 21) and eight (Chèv 1, 3, 4, 7, 8, 20, 21, 22) isolates respectively. Detailed molecular analysis revealed that VP7 sequence of the G10 caprine Moroccan isolate (RAV/Goat-wt/Mor/Chèv8/2012) showed maximum nucleotide similarity of 95% with the bovine strain B75/G10 isolated in India while the G6 Moroccan RVA/Goat-wt/Mor/Chèv21/2012/ presents a similarity of 91% with the human G6 isolate (RAV/Human-wt/BEL/810925/1997). Whereas sequence analysis of the gene encoding the VP4 of all strains determine their genetic relation to the guanaco (Lama guanicoe) RVA/Guanaco-wt/ARG/Chubut/1999/G8P[14] (96% of nucleotide similarity) and to a less extend (92% of nucleotide similarity) with the RVA/Vicuña-wt/ARG/C75/2010/G8P[14]. Our study demonstrates that G10 serotype was common to nomadic goat kids, suggesting a high frequency of RVA transmission between goats and cattle. In addition, the G6 type was also detected and, thus transmission between this species and human is probable. Our study reveal for the first time that Moroccan caprine P[14] rotavirus strains can be the result of interspecies transmissions from cameldids.

Oral 23 (short) and Poster 06 - Circulation of pig group A and C rotaviruses in Belgian diarrheic suckling pigs and its impact on veterinary diagnostical analyses

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**Keywords:** rotavirus A, rotavirus C, suckling pigs

**INTRODUCTION:**

Group A and C rotaviruses have been identified as important causes of diarrhea in suckling piglets, together with *Escherichia coli*, *Clostridium perfringens* and *Isospora suis*. Though, the importance of rotavirus infections on Belgian pig farms has not been investigated, which hampers the development of good strategies to diagnose and prevent this economical important disease.

**OBJECTIVES:**

In this study, it was aimed to investigate the presence of rotavirus A (RVA) and C (RVC) infections among Belgian diarrheic suckling pigs, in order to optimize current diagnostic...
strategies used in veterinary practice. Furthermore, the VP7 and VP4 genes of circulating strains were characterized, as this may benefit future vaccine formulation.

MATERIALS AND METHODS:
The presence of RVA, Escherichia coli, Clostridium perfringens and Isospora suis in diarrheic fecal samples (n=45) of suckling pigs less than 2 weeks old from 36 farms were investigated at a private diagnostic laboratory. However, veterinarians specified for which pathogens diagnostic tests should be performed. Here, RVA was diagnosed using a fast antigen detection strip, whereas bacteria were isolated on specific agars. Coccidia were purified using flottation, and visualised under a microscope. At the Laboratory of Virology, all samples were analyzed for RVA and RVC using RT-qPCR, and the genes encoding outer capsid proteins VP7 and VP4 were characterized by partial sequencing and phylogenetic analyses.

RESULTS:
Many of the common agents involved in the pathogenesis of diarrhea in suckling piglets were not routinely investigated in veterinary practice. However, in 61% of 36 farms tested, high viral loads of RVA (6.96 to 11.95 log10 copies/g feces) and/or RVC (5.40 to 11.63 log10 copies/g feces) could be detected, whereas rotavirus infections could only be diagnosed on 25% of the farms using a fast RVA antigen strip. Seventeen of these RVA strains were characterized, resulting in the detection of 4 different G-genotypes (G3, G4, G5 and G9) and 4 different P-genotypes (P[6], P[7], P[13] and P[23]) in 8 different G/P combinations. VP7 genotypes G5 and G4, and VP4 genotype P[7] were encountered most frequently (29.4% each). All RVC strains belonged to genotype G6 (VP7), except for one strain possessing the G1 genotype. Moreover, VP4 genes of Belgian RVC strains were genetically highly heterogeneous. Escherichia coli was also frequently isolated in the present study, but unfortunately the characterization of virulence factors was not requested routinely, making it difficult to interpret diagnostic results. Furthermore, most Clostridium perfringens strains were isolated from rotavirus negative samples. *I. suis* was only detected in 2 out of 45 samples, and probably underdiagnosed due to a lack of requests for routine testing in veterinary practice.

CONCLUSIONS:
As a conclusion, routine testing for RVA and RVC using RT-qPCR in diarrheic feces of suckling pigs is advised, but also diagnostic investigations of other pathogens should be carried out more frequently in order to come to sound conclusions, and to install durable and efficient prophylactic measures on affected pig farms.

Session V: Environment, molecular epidemiology and animal to human transmission

Oral 24 - Human rotavirus removal in a membrane bioreactor wastewater treatment process

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*Keywords: Human rotavirus, Wastewater treatment, Public health*

**INTRODUCTION:**
Rotavirus is the most common cause of gastroenteritis in children, and wastewater effluent discharge is the major source of human enteric viruses detected in the water environment. As these viruses are very resistant, it is important to ensure proper treatment of sewage to lower further contamination of environmental waters. Regarding this point, a membrane bioreactor (MBR) is a promising key technical element for wastewater treatment with several advantages such as high quality effluent and compactness. Virus removal in MBR processes keeps receiving attention due to the epidemiologically significant fact that pathogenic viruses in wastewater are highly diverse.

**OBJECTIVES:**
To evaluate removal properties of human rotaviruses in a full-scale MBR plant, their concentrations were monitored and compared to norovirus concentrations. We hypothesized that virus adsorption to mixed liquor suspended solids (MLSS) is significant for virus removal, and adsorptive behaviors of rotavirus and norovirus strains were investigated.

**METHODS:**
Untreated and treated wastewater samples (13 of each) were collected from October 2013 to May 2014 in a full-scale MBR plant. Analysis was performed on 40 ml of sample following the polyethylene glycol precipitation and nucleic acid extraction. To evaluate binding capacity of viruses to MLSS, a large excess of rotavirus G1P[8] and norovirus GI.1 and GII.4 strains from stools was added to mixed liquor samples. The mixture was continuously stirred and sampled after 1, 5, 10, 20, 30, and 60 min of virus inoculation. Unadsorbed viruses were recovered by filtering the mixture with 0.45-μm-pore-size membrane. Viral RNAs were extracted and concentrations were determined by real-time RT-PCR assays targeting the NSP3 gene for rotavirus A or the polymerase-capsid junction region for norovirus. Percentage of adsorption was calculated based on the inoculum and unadsorbed virus concentrations.

RESULTS:
Rotavirus A and norovirus were detected in 77% and 85% of untreated wastewater with mean concentrations of 5.7 and 6.2 log copies/L, respectively. In treated wastewater samples, rotavirus A was detected frequently (38%), whereas norovirus was once (8%). This suggests that rotavirus A was less efficiently removed in the full-scale MBR plant compared to norovirus. Adsorption experiments confirmed that norovirus GI.1 and GII.4 strains were efficiently adsorbed to MLSS after 60 min of reaction (96±3% and 96±4%, respectively). In contrast, rotavirus G1P[8] strain was less adsorbed to MLSS and larger variation was observed (65±29%).

CONCLUSIONS:
We speculate that different isoelectric points of viruses (8.0 for rotavirus SA11 and 5.5–6.0 for norovirus strains) is one of the factors contributing to the different adsorptive properties to MLSS, since surface charge and hydrophobicity play a major role in viral adsorptive behavior to solids. Effect of virus-particle association in untreated wastewater on viral binding to MLSS should next be investigated. Our observations suggest that rotavirus is less adsorbed to MLSS compared to norovirus, resulting in lower removal efficiency in the full-scale MBR plant.

Oral 25 - Mining and modelling; human and animal rotavirus epidemiology in Ireland

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Keywords: human rotavirus, porcine rotavirus, whole genome sequencing

INTRODUCTION:
Group A rotaviruses (RVs) are an important cause of gastroenteritis, in the young of human and animals. Rotaviruses are non-enveloped, triple layered viruses, with segmented genomes, which allows them to reassort (exchange genes during co-infections), resulting in novel strains, potentially capable of infecting humans and animals. There are considerable data on the genes encoding the surface proteins, VP7 (defining G-types) and VP4 (defining P-types), however, less is known about the genetic make-up of the remaining 9 gene segments of emerging and endemic RV strains. The frequency of gene reassortment occurring in nature is unknown, as relatively few animal RV genomes have been sequenced, so an understanding of the zoonotic risk is hampered by limited information on strains implicated in disease in various species.

OBJECTIVES:
An earlier project (led by our group) provided detailed analysis of the molecular epidemiology of RV in humans and food animals in Ireland, resulting in a large body of data accumulated over a long period of time. We have now established a large scale genomic project which will sequence the genomes of selected archived and recent isolates from human and porcine RVs, in collaboration with Dr Jelle Matthijnsens, Prof Anthony Staines, Dr John Morgan and Dr John McKillen.

METHODS:
Selected human samples from previous epidemiological studies, samples obtained from Cork University Hospital, and the Royal Victoria Hospital, Belfast were analysed. Archived RVA positive porcine samples were obtained from symptomatic animals, provided by Dr John McKillen, AFBI, Belfast and 100 samples taken from asymptomatic animals from a large commercial piggery in Southern Ireland. Selected human samples were G and P typed, and
representative samples were subjected to Sanger sequencing for confirmation. Due to lack of availability of reliable G and P typing protocols for porcines, the VP7 and VP4 genes were subjected to Sanger sequencing to elucidate the G and P types. Subsequently, selected samples were subjected to VLP purification in combination with Illumina sequencing to obtain complete genomes of RV and other viruses present at the Genomics Core Facility, KU, Leuven.

RESULTS:
Preliminary analyses indicate that the profile of the human samples contain typical human genotype constellations, with no evidence of mixed infections. As expected, from results of our previous studies, porcine samples contained a wide range of G and P types. Based on Sanger sequencing, samples were found to contain G2, G3, G4, G5, G11, in combination with P[6], P[13], P[32]. Representative samples were selected for Illumina analyses, resulting in the unexpected detection of a plethora of other viruses, e.g. Astrovirus, Enterovirus, Kobuvirus, Porcine associated stool circular virus, Rotavirus B, Sapovirus and Teschovirus.

CONCLUSIONS:
Our large-scale RV genomics projects provide insight into how RVs evolve during their spread through the human population and are of huge potential utility for development and improvement of diagnostic tests and vaccines, in human and veterinary medicine. The recovery of a wide range of other viruses from porcines warrants further research on the dynamic interaction between pathogens, overcrowding, social stress and environmental conditions in pig herds.

Oral 26 - Whole genome detection of rotavirus mixed infections in human, porcine and bovine samples co-infected with various rotavirus strains collected from sub-Saharan Africa.

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Keywords: Rotavirus, Mixed infections, Africa

INTRODUCTION:
Group A rotaviruses (RVA) are among the main global causes of severe diarrhoea in children under the age of five years. Mixed infections and untypeable RVA strains are frequently reported in Africa.

OBJECTIVES:
To gain insights into the whole genome diversity and evolution of rotaviruses deemed to be mixed genotypes and/or co-infections by conventional RT-PCTR and Polyacrylamide gel electrophoresis (PAGE).

METHODS:
We analysed rotavirus-positive human stool samples (n=13) obtained from hospitalised children under the age of five years who presented with acute gastroenteritis at sentinel hospital sites in six African countries, as well as bovine and porcine stool samples (n=1 each), using PAGE, conventional RT-PCR and Next generation sequencing.

RESULTS:
PAGE analyses and genotyping with G- (VP7) and P-specific (VP4) typing primers suggested that 13 of the 15 samples contained more than 11 segments and/or mixed G/P genotypes. Full-length amplicons for each segment were generated using RVA-specific primers and sequenced using the Ion Torrent and/or Illumina MiSeq next-generation sequencing platforms. Sequencing detected at least one segment in each sample for which duplicate sequences, often having distinct genotypes, existed. This supported and extended the PAGE and RT-PCR genotyping findings that suggested these samples were collected from individuals that had mixed rotavirus infections. We also report a unique genome segment 9 (VP7), whose G9 genotype belongs to lineage VI and clusters with porcine reference strains. Previously, African G9 strains have all been in lineage III. Furthermore, additional RVA segments isolated from humans have a clear evolutionary relationship with porcine, bovine and ovine rotavirus sequences, indicating relatively recent interspecies transmission and reassortment.

CONCLUSIONS:
Multiple RVA strains from sub-Saharan Africa are infecting mammalian hosts with unpredictable variations in their gene segment combinations. Whole-genome sequence
analyses of mixed RVA strains underscore the considerable diversity of rotavirus sequences and genome segment combinations that result from a complex evolutionary history involving multiple host species.

Acknowledgements: We thank the funding organisations; the National Institutes of Health (contract number HHSN272200900007C), the South Africa Medical Research Council, the Poliomyelitis Research Foundation and the National Research Foundation.

**Oral 27 - Genetic diversity of rotavirus A strains among children less than 5 years old with acute diarrhea in Mozambique**

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**Keywords:** Mozambican RVA, Prevalence, Genetic diversity

**INTRODUCTION:**
Rotavirus is the major etiologic agent causing viral gastroenteritis, infecting every year millions of children under five worldwide. According to the World Health Organization (WHO), it is estimated that globally, more than 500,000 deaths occurs each year among children as result of rotavirus infection. Mozambique will soon introduce the vaccine against rotavirus in the immunization program, however, very few data on epidemiology and genotypes circulation in the country is available.

**OBJECTIVES:**
The main objective of the present study was to determine the prevalence of rotavirus among children hospitalized with acute diarrhea and characterize the genotypes.

**METHODS:**
Between February 2012 and September 2013, stool specimens from 384 children less than 5 years of age hospitalized with acute diarrhea were collected in Mavalane General Hospital and Manhiça District Hospital both in the South region of Mozambique. The samples were tested for the presence of rotavirus A by ELISA using the ProSpecT TM Rotavirus kit (Oxoid, United Kingdom). Positive samples to rotavirus were then processed by multiplex RT-PCR in order to determine the G and P genotypes using the protocols previously described (Gentsch et al. 1992; Gouvea et al. 1990).

**RESULTS:**
Rotavirus antigen was detected in 163 (42.4%) stool samples. The average age of children with rotavirus was lower (11.5 months, Standard Deviation [SD] 8.03) compared to children without rotavirus (13.7 months, SD 8.90). When stratifying by age, the highest incidence of infection was observed in children between 0 and 5 months of age (p = 0.031). Of the 163 cases of rotavirus, 141 (86.5%) occurred in the dry season (from April to September) and 22 (13.5%) in the wet season (October to March). This difference was statistically significant (p <0.001). During the dry season, the peaks of detection were observed in July and August where the proportion of positive samples ranged 69-74%. It was possible to genotype for both G and P types 107 (65.6%) samples. The most prevalent G types were G2, 43/107 (40.2%) and G12, 41/107 (38.3%), while the predominant P types were P[4], 51/107 (47.7%) and P[6], 36/107 (33.6%). The predominant G/P combinations were G2P[4], 41/107 (38.3%) and G12P[6], 33/107 (30.8%). Other combinations found were G8P[4], G12P[P8] 5/107 (4.7%) each and mixed infections G12P[6]P[8], 2/107 (1.9%).

**CONCLUSIONS:**
The results of this study showed that rotavirus is implicated in high proportion of diarrhea in hospitalized children and highlights the diversity of rotavirus strains in Mozambique. Therefore, there is a need to establish a surveillance system for rotavirus across the country and monitor the genotypes in circulation, as they may have implications for the effectiveness of vaccine to be soon introduced.

**References**
Oral 28 - Rotavirus epidemiology in four districts of Angola before vaccine introduction

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4. Departamento do Controlo de Doenças (DCD) da Direcção Nacional de Saúde Pública (DNSP), Luanda, Angola.

INTRODUCTION:
Rotavirus (RV) is considered the leading cause of moderate-to-severe diarrhea in children under five years of age (<5) in developing countries, where more than 90% of the global death toll attributed to this virus occur. Angola is a sub-Saharan country with high mortality due to pediatric diarrhea. Objective: In the absence of epidemiological data on pediatric diarrhea, and anticipating the RV vaccine introduction in the country, the objective of the present study was to detect and characterize the RV circulating strains in children <5 with acute gastroenteritis attending the pediatric emergencies in four districts of the country.

METHODS:
Faecal specimens (n=343) were collected from eligible children in: Cabinda, Luanda, Huambo and Zaire districts. The sampling was done during dry season (June-August 2012) in six hospitals/health care centres (HCC) of Huambo and two HCC of Zaire and during wet season (September-October 2013) in one HCC from Luanda and one from Cabinda. Samples positive for RV by immunochromatographic rapid test were G and P typed by hemi-nested type-specific multiplex PCR, subgrouped for the VP6 gene by real-time or conventional PCR, the VP4 and VP7 genes from a subset of samples were further sequenced for phylogenetic analysis.

RESULTS:
A high detection rate (34.5%, 119/343) of RV was observed. G1 was the most common G-type (84%), whereas P[8] (51.3%) followed by P[6] (37.8%) were the most common P-types. The combination G1P[8] was identified in 50.4% of the RV positive samples followed by G1P[6] (28.6%). Genotypes such as G2P[4], G8P[6], G12P[6] and G9P[6] were also identified, though with lower frequencies (1%-5%). Nearly 7% of the samples were non-typeable by multiplex PCR or sequencing. A strong association of G1P[8], G1P[6], G9P[6] and G12P[6] strains with VP6 SGII was demonstrated, while G2P[4] and G8P[6] were associated to VP6 SGI. Phylogenetic analysis of selected samples showed that the VP7 and VP4 genes of G1P[8], G1P[6] and other RV strains from Angola clustered with corresponding reference genotype sequences with bootstrap values ≥99%. The P[6] genes belonged to two clusters; one sharing high nucleotide identity (99.8% nt identity) with G8P[6] strains from Democratic Republic of Congo and the other with G6P[6] strains from Burkina Faso (99.6% nt identity).

CONCLUSIONS:
The present study, the first to our knowledge from Angola, showed high prevalence of RV infection as well as the circulation of a high diversity of RV genotypes including the globally uncommon P[6] genotype in combination with various G types. These results underline the importance of RV surveillance in Angola, highly recommended to be continued after the vaccine introduction.

Acknowledgement:
We acknowledge the support of the DCD/DNSP from Angola. We are grateful to all children and their families for participation in this study and to the contribution of the medical and technical staff from the HCC to sample collection.
Oral 29 - Phylogenetic analysis of human group C rotavirus circulating in Brazil reveals a potential unique NSP4 genetic variant and high similarity with Asian strains

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Keywords: Gastroenteritis, phylogenetic analysis, Group C rotavirus

INTRODUCTION:
Group C rotaviruses (RVC) cause gastroenteritis in humans and animals worldwide, and the evidence for a possible zoonotic role has been recently provided.

OBJECTIVES:
To gain information on the genetic diversity and relationships between human and animal RVC, we sequenced the VP4, VP7, and NSP4 genes of 12, 19, and 15 human strains, respectively, detected in São Paulo state during historical (1988 and 1993) and recent (2007 and 2008) Brazilian rotavirus surveillance.

METHODS:
The RVC samples were collected during an over 30-year period of rotavirus monitoring in Brazil. During the annuals rotavirus surveillances, the fecal samples were first screened for Group A rotavirus (RVA), the most common rotavirus affecting humans, by commercial ELISA. RVA ELISA negative samples were further tested for the presence of RVC by SDS-PAGE, electronic microscopy, and RT-PCR for the VP6 gene. The genetic diversity of RVC was carried out by sequencing of VP7, VP6, VP4, and NSP4 genes.

RESULTS:
All RVC strains analyzed in the present study grouped into human genotype (G4-P[2]-E2). Phylogenetic analysis showed that RVC samples detected in 1988 and 1993 clustered together with strains from distinct continents, indicating that historical RVC strains circulating in São Paulo were closely related to those strains circulating worldwide. All three genes (VP7, VP4 and NSP4) of São Paulo RVC strains isolated in 2007-2008 exhibited close phylogenetic relationship with human RVC strains isolated in China and Japan. We identified two distinct clusters in the NSP4 phylogenetic tree. One cluster formed exclusively by human Brazilian strains detected in 1997 and 2003-2004 in Rio de Janeiro, Bahia, and Rio Grande do Sul states (Subgroup II) previously described in a different study, that displayed low sequence identities to other human strains formerly published, and to the Brazilian RVC strains (Subgroup I) characterized in the present study. These data suggests the circulation of two genetic profiles of the NSP4 gene in Brazil.

CONCLUSION:
In the phylogenetic analysis, we found two subgroups of NSP4 gene circulating in Brazil, divided into Subgroup I and II, making evident the simultaneous circulation of two distinct sublineages of RVC in the country. High sequence diversity in NSP4 gene was previously reported in Asia, and additional diversity in NSP4 RVC strains spreading in the world should be expected. In addition, Asian and Brazilian RVC strains are very closely related to each other, thus suggesting the occurrence of a single genetic variant in very different regions of the world. The existence of some temporal order genetic differentiation on the VP7, VP4 or NSP4 gene sequences was not observed. In this study all Brazilian RVC samples cluster with other human samples and did not show any evidence of animal ancestry. More in-depth molecular and epidemiological analysis of human RVC throughout the world will be needed to understand their diversity and clarify their evolution, as well as to develop classifications schemes.
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Oral 30 - Hospital-acquired rotavirus and norovirus gastroenteritis in Italian children, in 2014-2015

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Keywords: Nosocomial, Prevention, Transmission

BACKGROUND:
Hospital-acquired viral infections are a major concern for public health. Rotavirus (RV) and norovirus (NoV) are common causes of nosocomial viral acute gastroenteritis (AGE) in pediatric hospitals. Nosocomial AGE causes prolonged hospital stay, which increases both direct and indirect costs for the public health system and risk of death in preterm neonates. In Italy, RV was previously reported to account for 31-87% of nosocomial infections, followed by NoV (10-16.2%).

OBJECTIVES:
To investigate further the role of RV and NoV in hospital-acquired AGE and possible transmission routes of infection, we studied 39 cases of nosocomial AGE occurred between May 2014 and February 2015 at the Pediatric Hospital “Bambino Gesù” (OPBG) of Rome.

METHODS:
The patients enrolled were aged between 2 month and 12 years of age, and were admitted to the OPBG with a diagnosis other than GE. Patients considered in the study showed symptoms of acute diarrhea (liquid stool and >3 evacuations in 24 hours) after at least 48 hours of hospitalization and up to 24 hours after discharge. AGE caused a 2-day extra-length of hospital stay. RV G- and P- genotypes were defined following EuroRotaNet protocols, and samples were further tested by RT-PCR using primer sets for NoV detection and genogroup identification. Nucleotide sequencing and phylogenetic analyses were used to characterize viruses. The role of the environment, visitors and healthcare professionals in virus transmission was investigated matching clinical and epidemiological data collected with molecular data. The burden of nosocomial AGE associated to viral infections was described according to: frequency, epidemiology and clinical characteristics (Gorelick S, risk factors, seasonality, incidence).

RESULTS:
Thirty-one of 39 cases analyzed resulted positive for group A rotavirus (RVA). The predominant genotype was G4P[8] (19/31 cases) followed by G1P[8] (3/31), G3P[8] (2/31), and G9P[8] (1/31). A high rate of mixed RVA infections was also detected (7/31, 22.6%). Nineteen samples resulted positive for NoV, and GII.4 was revealed by sequence analysis to be the predominant genotype involved. Fourteen samples gave positive result for both RVA and NoV.

CONCLUSIONS:
This study confirms the predominant role of viral infections in the etiology of nosocomial AGE. Analysis of virological and epidemiological data will help to clarify the origin of infection in order to activate effective prevention and control measures, including RV vaccination. In-depth investigations of virus nosocomial AGE may elucidate the epidemiology, persistence and transmission mechanisms of enteric viruses spread in hospital settings.
Oral 31 - Patterns of rotavirus strain circulation across twelve European countries prior to the introduction of routine rotavirus vaccination – 2007/08-2012/13

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Keywords: Epidemiology, Strain type

BACKGROUND:
The EuroRotaNet surveillance network established in 2007 and including 16 countries has enabled rotavirus strain diversity across Europe to be monitored. Critically, the availability of substantial strain typing and epidemiological data for EuroRotaNet countries provides a baseline for strain diversity and case epidemiology prior to vaccine introduction. We investigated “in” and “out”-of-rotavirus season differences in circulating strain types and case age for twelve countries in Europe prior to routine / wide spread vaccination.

METHODS:
A short semi-structured questionnaire was distributed to EuroRotaNet country leads to identify additional detail on inter and intra country testing practices. We used this information to inform data handling and support interpretation of the epidemiological analysis. Using strain type and epidemiological data from twelve EuroRotaNet countries (without routine / widespread vaccination) for six rotavirus seasons spanning September 2007 to August 2013 we assessed the variance of circulating strain types and age of infection “in” and “out”-of-peak rotavirus season. We defined “In” and “out”-of-peak rotavirus season for each country using country specific thresholds based on median weekly specimen frequency and data gathered from the testing practice survey. Age groups were constructed and strain types were grouped into G1P[8], genotype 1 (Wa-like: excluding G1P[8]), genotype 2 (DS-1-like), mixed and untypable, and other. Multivariate logistic regression analysis was conducted to examine relative strain type circulation “in” and “out”-of-peak-season. Age and year of specimen adjusted odds ratios were calculated for each country.

RESULTS:
G1P[8] was the dominant strain type (48%) in 10/12 countries studied. Multivariate analysis showed that relative proportions of strain types changed significantly “in” and “out”-of-peak-season in 10/12 countries. Bulgaria, France, Italy, the Netherlands, Spain, and the United Kingdom experienced a relative decline of G1P[8] “out” of peak season and a proportionally representative increase in other strain types. The majority (9/12) of countries experienced significant relative increases in circulation of rarer strain types “out”-of-season. Age of case infection varied significantly “out”-of-season compared to “in”-season in 6/12 countries. In particular the UK showed distinct variation with the number of specimens from <11months old increasing from 34% “in”-season to 39% “out”-of-season (adjusted Odds Ratio [aOR] 1.7; 95% CI 1.2-2.3) and 5+ year olds increasing from 9% to 17% (aOR 2.5; 95CIs 1.7-3.8).

CONCLUSIONS:
Our findings show significant country specific differences in strains circulating during the peak rotavirus season and “out” of season and variation in age of cases “in” and “out”-of-peak-season was shown in some countries. Potentially protection built in the highest incidence age-group “in”-season results in a reduction in the number of susceptible hosts “out”-of-season enabling a relative increase of infection in other age-groups. Furthermore, the decline of predominant strain types “out”-of-season could be due to homotypic resistance generated “in”-season reducing the number of susceptible hosts, whilst rarer strains may increase “out”-of-season due to greater resistance and persistence in the environment in warmer conditions. These results should be interpreted with caution as the country specific nature of these results make identifying a generalised explanation difficult.

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Keywords: Evolution, Italy, G4P[8]

BACKGROUND:
Group A rotaviruses (RVA) are the leading cause of acute gastroenteritis (GE) in young (<5 years of age) children, causing up to 450,000 deaths worldwide, mostly in developing countries. RVA virions are icosahedral, triple-layered and non-enveloped particles possessing a segmented genome made of 11 double-stranded RNA linear segments. The RVA outer layer is composed of the two major viral proteins VP7 and VP4, encoded by gene 9 and 4, respectively. VP7 (G-type) and VP4 (P-type) genotypes are the basis for the binary RVA nomenclature. Although at least 27 G-types and 37 P-types of rotavirus are presently known, most of RVA infections in humans worldwide are related to five major G/P combinations: G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8].

OBJECTIVES:
During the Italian 2013 surveillance season of RVA gastroenteritis, a total of 1112 samples were collected from hospitalized symptomatic children, and were genotyped Most analyzed strains belonged to the five major human genotypes, and 88 samples (7.9%) presented the G4P[8] genotype combination. Among these common strains, 22 G4P[8] RVA strains, from different Italian regions, were subjected to nucleotide sequencing of genes coding for VP4, VP6, VP7 and NSP4 proteins in order to confirm the genotyping results and to obtain information on the evolution of these common RVA strains.

METHODS:
RVA genotyping was performed by reverse transcription nested polymerase chain reaction (RT-nPCR), following EuroRotaNet protocols. Nucleotide sequencing of the VP7, VP4 (VP8*), VP6 and NSP4 genes amplified was performed with the same primers used for the RT-PCR, using the BigDye chemistry. The phylogenetic tree construction was performed with MEGA6, applying the Maximum-Likelihood (ML) method. The VP4 (VP8*) deduced amino acid sequences were used to build the protein prediction structures, using the ModWeb tool.

RESULTS:
The phylogenetic analysis showed that the Italian G4P[8] RVA strains analyzed belonged to lineage G4-I for VP7 and to lineage P[8]-III, for VP4, in line with the currently circulating G4P[8] strains detected in children worldwide. All the VP6 and NSP4 analyzed sequences belonged to genotype 1. The phylogenetic trees revealed high nucleotide identity between the G4P[8] RVA strains detected in this study and G4P[8] strains detected previously in Europe, Asia and Africa, revealing the formation of at least three separate intra-lineage evolutionary clusters for VP4, VP6 and NSP4, suggesting the presence of imported exotic NSP4 genes for at least five of the strains analyzed. The analysis of the deduced amino acid sequences of the outer capsid proteins belonging to the Italian G4P[8] RVA strains revealed the presence of substitutions within the hypervariable antigenic regions investigated.

CONCLUSIONS:
Continuing surveillance of RVA strains is necessary to investigate the evolution of both common and uncommon rotaviruses causing diarrhea in humans. Molecular analysis of strains that belong to the five major human genotypes can improve the knowledge on the epidemiology of this pathogen, providing data for assessing the effectiveness of anti-RV vaccine campaigns.
Oral 33 - Rotaviruses in hospitalized children with diarrhoea in Slovenia – still the leading cause

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Keywords: rotavirus epidemiology, hospitalised children, diagnostics

INTRODUCTION:
Group A rotavirus is still the leading cause of acute infantile diarrhoea at least in countries, where low rotavirus vaccine coverage is noted or vaccine is not implemented in the vaccination schedule. In Slovenia, rotavirus vaccine is available since 2007 and the vaccination coverage is 24%. Thus, it is important to maintain the strain surveillance and molecular characteristics of GARV in the future.

OBJECTIVES:
The aim of the study was to analyse the burden of rotavirus infections in hospitalized children in Slovenia with low vaccination coverage, and compare it to other important enteric pathogens. Also, rotavirus antigen detection method and electron microscopy, currently in use in our laboratory, were compared to the rotavirus molecular methods in order to evaluate the clinical limitations of those methods.

METHODS:
Stool samples from 359 hospitalized children were collected from October 2011 to October 2012. A 10% suspension was prepared and examined for the most important viral, bacterial and parasitic pathogens using real-time (RT)-PCR. In addition, stool samples were screened with antigen detection method (ELISA) for group A rotavirus and electron microscopy. The data on viral, bacterial and parasitic co-infections were examined in detail on the basis of Cq values of the (RT-)qPCR to estimate the role of specific pathogen in co-infections.

RESULTS:
In total, 297 stool samples were examined for enteric pathogens, of which rotavirus was the most prevalent, found in 55.6% (165/297). The list of other detected pathogens was as follows: parechovirus 16.2%, norovirus GGII 14.8%, E. coli 11.7%, *Clostridium difficile* 8.1%, adenovirus species F 7.4%, astrovirus 5.4%, *Campylobacter spp.* 5.1% and others with the prevalence below 5%. Rotavirus as a single pathogen was detected in 58.18% (96/165) of rotavirus positive samples. As co-detected pathogen mostly parechovirus (18/69), pathogenic *Escherichia coli* (16/69) and toxin producing *Clostridium difficile* (9/69) were found. Overall, parechovirus was detected in 16.2% of stool samples and only in 4 cases (1.3%) as a single pathogen. In multiple infections it was observed with lower abundance compared to co-detected pathogens. Comparing rotavirus ELISA and EM with rotavirus RT-qPCR it was observed, that ELISA and EM sensitivity is good enough for accurate diagnostics of acute rotavirus infection in hospitalized children. According to the Cq values of the RT-qPCR, the cut-off for rotavirus was set to 25. Above the cut-off rotavirus was mostly found with other pathogens, which were more abundant according to the Cq values. In this study rotavirus genotype G1P[8] was the most prevalent (45.19%), followed by G2P[4] (24.44%), G9P[8] (14.07%) and G4P[8] (11.85%).

CONCLUSIONS:
In Slovenia rotavirus still remain the most important pathogen in hospitalized cases of acute gastroenteritis, representing more than half of hospitalized diarrhoea cases. Although rotavirus was found in multiple infections in 41.82% of positive samples, its abundance was higher, compared to co-detected pathogens. Parechovirus was most often detected as co-pathogen, probably with minor or no role in the disease. EM and ELISA are sensitive enough for rotavirus diagnostics and cut-off should be considered in real-time PCR to avoid detection of asymptomatic rotavirus infection.
Oral 34 (short) & Poster 12 - Surveillance of rotavirus strains in Valencia, Spain, during 12 years (2003-2014)

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Keywords: Rotavirus, surveillance, epidemiology

INTRODUCTION:
Group A rotavirus (RVA) is the leading cause of severe diarrhoea among infants and small children worldwide. Our laboratory contributes to monitor the distribution of rotavirus genotypes in Spain, collaborating with the European Rotavirus Surveillance Network (EuroRotaNet). Objective: To analyse the evolution of RVA genotypes along the years, before and after vaccine implementation.

METHODS:
Rotavirus infection was diagnosed by immunochromatographic (Certest Biotec) or ELISA (Meridian Bioscience, Inc.) tests used in the daily routine of our hospital. G and P genotypes were characterized in a total of 1,470 strains. RNA was extracted from 10% fecal suspensions by the guanidinium isothiocyanate/silica or by using Trizol reagent (Life Technologies). RVA genotyping was performed by multiplex RT-PCR followed by agarose gel electrophoresis, according to the standard procedures of the EuroRotaNet project (http://www.eurorota.net).

RESULTS:
As a whole, the distribution of G/P types along 12 years have been: G1P[8] 53.2%, G9P[8] 11.5%, G2P[4] 8.3%, G3P[8] 5.6%, G4P[8] 2.1%, G12P[8] 6.9%. Mixed infections with two or more genotypes were observed in 5.9% of the samples. G1P[8] was the predominant genotype in seven rotavirus seasons, in 2003-04, 2004-05, and during five consecutive seasons from 2007 to 2012. A sudden emergence of G9P[8] occurred in 2004-05, becoming G9 the predominant genotype in 2005-06 (84.6%) and in 2006-07 (68.3%). After being almost absent, G9P[8] re-emerged in 2010-11, representing 37% of all strains in 2012-13 and 45.4% in 2013-14. G12P[8] was detected in Valencia in the 2011-12 epidemic season and represented 29.6% of all typed strains in 2013-14. After introduction of rotavirus vaccines in 2007, a temporary increase of G2P[4] was observed in 2008-09 (18.8%) and in 2009-10 (20.7%). Uncommon RVA genotypes detected throughout the consecutive seasons have been G8P[6] (representing 5.3% of typed strains in 2008-09), G6P[14] (1 strain in 2007-08), G8P[14] (1 strain in 2009-10), G8P[4] (3 strains in 2010-11), and G3P[14] (1 strain in 2010-11). Whereas G12 strains belong to genogroup 1 (Wa-like), all G8 strains belong to genogroup 2 (DS-1-like).

CONCLUSIONS:
Rotavirus surveillance clearly shows the fluctuation of genotypes from season to season. Some emerging genotypes can persist circulating for years while others disappear in a short time.


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Keywords: Surveillance, Rotavirus

INTRODUCTION:
Rotavirus is the major cause of acute gastroenteritis in infants worldwide. Despite most of the 450,000 annual deaths worldwide occur in developing countries, morbidity is high also in industrialized countries, calling for universal use of vaccines. In Italy, the Istituto Superiore di Sanità of Rome (ISS) has implemented a nationwide laboratory-based surveillance of acute rotavirus gastroenteritis, to investigate the diversity of rotavirus strains circulating before the introduction of large-scaled vaccination. RotaNet-Italy is linked to the EuroRotaNet network, including 17 European diagnostic laboratories.
OBJECTIVES:
This study was aimed to investigate the circulation of different rotavirus genotypes in Italy, describing geographic and temporal variations in the predominant viral types, and to detect the possible emergence of uncommon rotaviruses of animal or exotic origin.

METHODS:
From January 2007 to August 2014, approximately 9330 rotavirus positive stool samples were collected from pediatric patients with acute diarrhea hospitalized in 14 Regions throughout the Italian territory. After viral RNA extraction, samples were genotyped for VP7 (G-type) and VP4 (P-type) genes by reverse transcription and multiplex-PCR, using type-specific primers, in accordance with the EuroRotaNet methods and algorithm.

RESULTS:
Significant variation in the frequency of different rotavirus genotypes was observed between different years and areas of Italy. Most strains belonged to genotypes G1-G4, and G9, associated with either P[8] or P[4], commonly found in humans worldwide. Overall, the most common rotavirus genotype detected during the seven rotavirus seasons was G1P[8] (51%), followed by G9P[8] (16%), G4P[8] (10%), G2P[4] (8%) and G3P[8] (3%). However, in at least 3% of cases, unusual or novel strains, such as G3P[19], G6P[6], G6P[9], G8P[4] and G12P[8], were also detected, suggesting either gene reassortment events between rotaviruses of different origin or importation of strains from other countries. In particular, during the surveillance 2012-13 the diffusion of the emerging G12P[8] rotavirus genotype was unexpectedly detected in the Central Italian region of Umbria (6%), and in different regions during the following season 2013-14 (7%). Mixed infections with two or more rotavirus strains were observed frequently (8% of patients). Further characterization of strains by partial sequence analysis was carried out to evaluate the genomic evolution of viruses. Most rotavirus infection occurred in children <2 years of age, but cases were also reported in older subjects, identifying risks of infection through contact with infected children and increased susceptibility of the elderly population to rotavirus.

CONCLUSIONS:
Data from seven-year RotaNet-Italy surveillance confirm the genetic diversity of rotaviruses circulating in Italy, and the existence of remarkable differences between Regions and years. Although overall data confirm the suitability of present vaccine formulations, novel strains are also shown to emerge occasionally in Italy, which may unveil possible reassortment and/or zoonotic transmission. Rotavirus surveillance is valuable to investigate the diversity of rotavirus strains circulating and to control possible emergence of novel strains.

Full list of members is present at: http://www.iss.it/criv/index.php?lang=1&id=363&tipo=9

Oral 36 (short) & Poster 14 - Intra-genotypic characterization of group A rotavirus strains circulating in Germany (2008-2013)

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Keywords: phylogenetic analysis, intra-genotypic characterization, strain distribution

INTRODUCTION:
Group A Rotavirus (RVA) infections are still the most frequent cause of acute gastroenteritis among infants in Germany, despite the availability of vaccines. However, the number of reported RVA infections in Germany has decreased from 80,000 in 2007/8 to 33,000 cases in 2013/4. The influence of vaccination on circulating RVA strains remains unclear. Seasonal and regional distribution of RVA strains has been very heterogeneous. Therefore, continuous comparison of sequencing data from circulating RVA strains is needed to distinguish seasonal or regional fluctuation bias from possible global trends that could have an influence on vaccine efficacy.

OBJECTIVES:
Sequencing and intra-genotypic analysis of circulating RVA strains to identify possible trends that are shared among samples from different regions or seasons.

METHODS:
In addition to G and P typing, a subset of samples with common RVA genotypes (G1, G2, G3, G4 and G9) collected in different regions of Germany was further characterized by sequencing
and phylogenetic analysis of VP7, VP4 and NSP4. These data were analysed with respect to genotype distribution and intra-genotypic differences.

RESULTS:
During 6 consecutive seasons (2007/8 to 2012/13), G and P typing data from >3000 samples and sequencing data (VP7, VP4, NSP4) of >10% of these samples were retrieved. By phylogenetic analysis, specific intra-genotypic patterns with respect to seasonal and regional distribution of some RVA sub-lineages or strains were observed.

CONCLUSIONS:
Despite of a heterogeneous mixture of co-circulating strains, different distribution patterns could be found, that are useful to monitor trends and identify possible emerging strains or beneficial strain characteristics.

Session VII: Emerging rotavirus strains

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INTRODUCTION:
Burkina Faso was one of the first African nations to introduce pentavalent rotavirus vaccine (RotaTeq) to its national immunization program in November 2013. The vaccine has yet to achieve widespread coverage and vaccine effectiveness has not been evaluated till date. Previous studies have shown a relatively high presence of globally rare genotypes such as G6 and P[6] which may have an effect on vaccine efficacy.

OBJECTIVES:
This study describes the molecular epidemiology of rotavirus among children less than five years of age in Ouagadougou, Burkina Faso, from December 2012 to November 2013, just prior to implementation of the rotavirus vaccination program.

METHODS:
Stool specimens, demographic and clinical information were collected from 154 children admitted to hospital due to diarrhea during December 2012 to November 2013. Rotavirus antigen was detected using ELISA and positive samples were genotyped by sequencing and/or multiplex semi-nested PCR.

RESULTS:
Overall, 42% (n=64) of samples were positive for rotavirus, the vast majority detected during the cold dry season (Dec-Feb). The predominant G genotypes were G12 (56%) and G6 (33%). The predominant G/P combination was G12P[8] (47%) and G6P[6] (30%). Also G2P[4] (n=3), G12P[6] (n=3) and G6P[8] (n=1) were detected. The G12P[8] strains dominated during the beginning of the rotavirus season (Dec-Jan), where after G6P[6] emerged. The G6 strains infected younger children (mean 5.7 months, range 1-12) whereas G12 strains infected children of all age-groups (mean 8.8 months, range 2-27; p<0.01). The VP7 gene of the G6 strains shared ~99% nt identity to the human G6 strains detected in Burkina Faso in 2010. Ongoing whole genome analysis indicate that the G6P[6] and G6P[8] strains share DS-1 like backbone and the VP7 gene of the G6P[8] strain clustered separately from the G6P[6] strains detected in this study. This indicate separate evolution and transmission from a previous reassortment event between a human G6P[6] and a P[8] strain. The VP7 gene of G12P[8] strains belonged to lineage III and shared high nt identity with G12 strains detected in Cameroon and Nigeria in 2010. Of note, the VP7 gene of the G12P[6] strains had 5% nt difference to the G12P[8] strains showing 2 variant VP7 G12 genes to be in circulation simultaneously.

CONCLUSION:
To conclude, this study shows predominance of both the globally emerging G12P[8] and the previously unusual G6P[6] strain. The G6P[6] strains emerging in Burkina Faso in 2010 now seem established as a regionally important rotavirus genotype. The detection of a human
G6P[8] genotype for the first time in Africa further highlight the ongoing evolution of the G6 strains with a potential for global spread. Continued studies are needed to establish vaccine effectiveness against these partially or fully heterotypic strains in the region.


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**Keywords**: Rotavirus, G12 genotype, evolution

**INTRODUCTION:**
Group A rotaviruses are one of the leading causes of acute gastroenteritis in young children worldwide. Rotavirus displays a seasonal pattern of infection in countries with temperate climate, with epidemic peaks occurring in winter and spring. Rotavirus vaccination may influence on the fluctuations of circulating rotavirus genotypes, especially in areas with low vaccine coverage. Significant annual changes in genotype distribution have been frequently detected even in the pre-vaccine era.

**OBJECTIVE:**
To survey the emergence and spread of G12P[8] rotavirus strains in different regions of Spain.

**METHODS:**
We participate in the European Rotavirus Network, EuroRotaNet, which was established in January 2007 to perform molecular epidemiology surveillance of rotavirus strains by characterizing their G and P types. Uncommon strains of epidemiological importance are further characterized by analysing the subgroup (VP6) and NSP4 genotype or by whole genome sequencing.

**RESULTS:**
A notorious emergence of G12P[8] strains was detected in the Basque Country (Northern Spain) in 2004-05, being the predominant genotype in the 2010-11 (65% of all strains) and 2011-12 seasons (81.6%). Whereas the prevalence of this genotype declined in the Basque Country to very low levels in 2012-13 (1.2%) and 2013-14 (2.3%), an increase of G12P[8] strains was detected during 2013-14 in other Spanish regions (Castilla-León, Aragón, Catalonia and Valencia), accounting overall for 15.3% of 466 typed strains. During the 2013-14 season, G12P[8] strains were detected in Valencia in 27.5% of rotavirus-positive samples. Phylogenetic analyses of the VP7 and VP4 genes demonstrated that they belong to lineages III of both G and P types. These strains display the typical human Wa-like gene constellation, and this may be the key to their recent increase and spread.

**CONCLUSIONS:**
Rotavirus G12[P8] should be considered as an emerging genotype in Spain, causing seasonal epidemics like the common human rotavirus genotypes G1–G4 and G9. After its emergence, G12[P8] genotype distribution fluctuates year to year and across different geographic regions. Continued surveillance of circulating rotavirus strains will reveal the future evolution of this genotype.

GIAMMANCO GM1, BONURA F1, SAPORITO L1, DI BERNARDO F2, CASCIO A3, FERRERA G4, COLLURA A1, TERRANOVA DM1, VALENZISE M1, ALLU MT5 and DE GRAZIA S1

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Keywords: Rotavirus, G12, Italy

INTRODUCTION:
Over the last few years a growing number of reports have signaled the detection of rotavirus G12 strains in humans worldwide, both in developed and developing countries, either sporadically or with remarkable incidence. Therefore, G12 strains are now considered to be the sixth most prevalent human RVA VP7 genotype, predominantly exhibiting either the P[6] or P[8] VP4 genotype and, less commonly, the P[4] and P[9] specificity. In Sicily, continuous surveillance of HRV circulation was carried on in Palermo since 1985 and in Messina since 2009 but no G12 strains were detected until 2012.

OBJECTIVES:
The present study has the aim to describe the first sustained circulation of rotavirus G12 in Sicily and to evaluate the genetic diversity of such strains.

METHODS:
The presence of rotavirus was investigated in 1647 stool samples collected from children (<5 years of age) admitted for acute gastroenteritis to three Sicilian Hospitals in Palermo, Messina and Ragusa during three consecutive years, from 2012 to 2014. The rotavirus isolates were G- and P-typed and the genetic diversity of the G12 strains was investigated by phylogenetic analyses of the VP7 and VP4 genes.

RESULTS:
Rotavirus infection was detected in 29.7% of the samples tested. In all the three hospitals independently of the year of sampling G1P[8] was the predominant strain affecting children (75% of the typed specimens). Interestingly, in 2012 G12P[8] was first detected in Palermo and represented the second most frequent genotype in Messina (20% prevalence). Thereafter, G12 strains continued to circulate in Sicily, showing a remarkable prevalence in 2013 in Ragusa (27.8%) and in 2014 in both Palermo (23.7%) and Messina (16.6%). The genetic diversity of the G12 strains detected in Sicily was investigated by phylogenetic analyses of the VP7 and VP4 genes. Among the four VP7 lineages of G12 rotaviruses described in literature, all but one of the Italian strains belonged to lineage III, but segregated into two different clusters (a and b). A single strain from Messina clustered in VP7 lineage II. Sequence analysis of the VP4 gene demonstrated that all the G12 strains belonging to VP7 lineage III, independently of the cluster (a or b), showed a P[8] genotype, while the lineage II strain had a P[9] genotype.

CONCLUSIONS:
The detection of rotaviruses with G12 specificity in this study represents the first report of such strain in Sicily. According to the latest EuroRotaNet report, G12P[8] strains increased significantly from 2006 onwards and reached considerable levels in a few European countries, although seasonal epidemics and local outbreaks have been generally reported. In Sicily, two different clusters of G12P[8] strains (lineage III-cluster a and -cluster b) and a single G12P[9] strain have been detected indicating the introduction in the island of at least three G12 clones. Apparently, the Sicilian G12P[8] strains of cluster III-b did not spread efficiently in this settled population since they disappeared after 2012. Conversely, G12P[8] of cluster III-a seems to have been able to reach sufficient fitness to stably circulate during the three years study.
Oral 40 - Complete genome analysis of a G12P[9] reassortant strain brings to the attribution of novel VP1, VP2, VP3 and NSP2 genotypes

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Keywords: Rotavirus, G12P[9], Novel genotypes

INTRODUCTION:
Group A rotaviruses (RVA) of genotype G12 are currently recognized as a globally emerging genotype. G12 strains have been demonstrated to be strongly versatile due to reassortment events, which may have contributed to their capability to spread in the human population. In Sicily, South of Italy, G12 RVAs, were first detected by the ISGEV infantile gastroenteritis surveillance system in 2012, more often in association with the P[8] and only in one case with the P[9] genotype. G12P[9] strains are uncommon strains and probably originated by multiple reassortments events introducing single segments on AU-1 backbone.

OBJECTIVES:
The aim of the present study is to determine the genetic constellation of the uncommon G12P[9] strain ME848/12 by full-length genome analyses, in order to investigate the origin of such strain.

METHODS
The complete genotype constellation of ME848/12 strain was determined and phylogenetic analyses were performed by MEGA 6 software.

RESULTS:
Based on the nucleotide sequence identities and according to the novel phylogenetic genotype classification system proposed by Matthijnssens et al., the G12-P[9]-I17-R12-C12-M11-A12-N12-T7-E6-H2 genetic constellation was assigned to the ME848/12 strain. This genes combination did not correspond to any of the established rotavirus genetic backbones and included segments of different animal origin. Phylogenetic analyses revealed that the VP1, VP2, VP3 and NSP2 genes of the Italian strain were distant from all established genotypes. Therefore, the Rotavirus Classification Working Group (RCWG) assigned them new genotypes R12, C12, M11 and N12. The nucleotide identity percentages of the 11 genes of RVA/Human-wt/ITA/ME848/12/2012/G12P[9] compared to cognate sequences recovered from GenBank are shown in table 1.

CONCLUSIONS:
Strain ME848/12 shows a puzzling genome the genes combination of which is probably the product of multiple reassortment steps involving both human and animal strains. Epidemiological surveillance involving full genomic analyses of G12 strains will be paramount to identify strains and/or reassorted genes that might be correlated with the genome of the G12 strain we detected in Sicily in order to evaluate whether it might represent a novel backbone for G12 rotaviruses.

Table 1. Nucleotide identity of the 11 genome segments of the Italian G12P[9] strain ME848/12 against prototype RVA strains and closest matches recovered from GenBank.

<table>
<thead>
<tr>
<th>Gene encoding</th>
<th>Cut-off % value for genotype assignation</th>
<th>Nucleotide identity % to</th>
<th>Genotype assigned</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Closest prototype strain (Strain name/Host origin)</td>
<td>Closest GenBank match (Strain name/Host origin)</td>
<td></td>
</tr>
<tr>
<td>VP7</td>
<td>80</td>
<td>91.3 (L26/Human)</td>
<td>98.6 (Arg720/Human)</td>
</tr>
<tr>
<td>VP4</td>
<td>80</td>
<td>88.9 (AU-1/Human)</td>
<td>97.7 (T152/Human)</td>
</tr>
<tr>
<td>VP6</td>
<td>85</td>
<td>92 (N5/Rabbit)</td>
<td>None cut-off</td>
</tr>
<tr>
<td>VP1</td>
<td>83</td>
<td>None above cut-off</td>
<td>None above cut-off</td>
</tr>
<tr>
<td>VP2</td>
<td>84</td>
<td>None above cut-off</td>
<td>None above cut-off</td>
</tr>
<tr>
<td>VP3</td>
<td>81</td>
<td>None above cut-off</td>
<td>None above cut-off</td>
</tr>
<tr>
<td>NSP1</td>
<td>79</td>
<td>97.3 (T152/Human)</td>
<td>97.7 (Arg721/Human)</td>
</tr>
<tr>
<td>NSP2</td>
<td>85</td>
<td>None above cut-off</td>
<td>None above cut-off</td>
</tr>
<tr>
<td>NSP3</td>
<td>85</td>
<td>95.6 (UK/Bovine)</td>
<td>95.6 (RV277/Pig)</td>
</tr>
<tr>
<td>NSP4</td>
<td>85</td>
<td>97.7 (N26/Human)</td>
<td>98.5 (RV176/Human)</td>
</tr>
<tr>
<td>NSP5</td>
<td>91</td>
<td>88.7 (DS-3/Human)</td>
<td>96.5 (N5/Rabbit)</td>
</tr>
</tbody>
</table>

*Novel genotype
Oral 41 (short) & Poster 16 - Transient emergence of G12 rotaviruses in French infants

de ROUGEMONT Alexis, KAPLON Jérôme, FREMY Céline, AHO-GLELE Ludwig Serge, POTHIER Pierre, The French Rotavirus Network
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Keywords: diarrhea in infants, G12 rotavirus emergence, clinical characterization

Group A rotavirus (RVA) are the leading cause of acute gastroenteritis (AGE) in young children worldwide. To follow up vaccine introductions, dedicated surveillance networks have been set up for investigating virological and clinical features of rotavirus infections. From 2009 to 2013, RVA-positive stool samples were collected from 3688 children under 5 years old admitted to the paediatric emergency units of 13 French large public hospitals. The genotyping of 3434 rotaviruses showed that G1P[8] strains (64.4% [55.3-73.2]) were predominant. G3P[8] (9.5% [1.5-19.0]), G9P[8] (8.3% [4.6-11.3]) and G2P[4] (8.1% [4.2-17.3]) strains had very changing incidence depending on seasons and regions, whilst G4P[8] (3.1% [0.8-7.2]) strains were mostly circulating locally. G12P[8] (2.1% [0-4.3]) strains emerged during the last two seasons with a prevalence of 4.3%. Most strains were associated with P[8] (89.2% [77.0-93.9]). Overall, 33 possible zoonotic reassortants (1.0% [0.4-1.5]) were also detected, such as G6 (27.2%) and G8 (18.2%), and were often associated with P[6] (69.7%). Among them, 2 G8P[8] strains were detected showing active recombination of bovine G8 with human P[8] strains, a first plausible step to human adaptation. Analysis of the clinical records of a group of 624 hospitalized children showed no difference in clinical manifestations in relation to genotype. Similarly, severity scores from 286 children show no difference in severity in relation to genotype. Among them, infection was severe in 51.7% of cases, but significantly less severe in children under 6 month old (P<0.0001). The relative stability of RVA genotypes currently co-circulating and the large predominance of P[8] type strains may ensure vaccine effectiveness in France. Establishing RVA genotypes remains however a key issue in understanding of the mechanisms by which strains emerge or are maintained in the population. The surveillance of rotavirus infections during ongoing and future vaccination programs will continue to monitor the emergence of new reassortants that may not respond to current vaccines and to help optimizing appropriate vaccine strategy against rotavirus disease, the more so as all genotypes can cause severe infections in infants.

Session VIII: Vaccine effectiveness and epidemiology

Oral 42 - Large increase of rotavirus diarrhea in the hospital setting associated with emergence of G12 genotype in a highly vaccinated population in Nicaragua

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Keywords: G12 rotavirus, Rotavirus vaccine, anti-rotavirus IgA

Rotaviruses (RVs) are a major cause of severe diarrhea in young children. Nicaragua introduced routine immunization with the pentavalent rotavirus vaccine (RV5) in 2006; which greatly reduced the incidence of diarrhea. A remaining concern has been the possible emergence of new RV strains to which the vaccination has less effect. In this study, 837 children with diarrhea in hospital settings were investigated for RV between May 2011 and July 2013. RVs were subsequently typed by multiplex PCR and/or sequencing. Fecal anti-RV IgA titers for a subset of RV-infected (n = 137) and non-infected children (n = 52), were determined with an in-house ELISA assay. The RV detection rate was 8% in 2011, followed by a sharp increase to 29% in 2012 and 19% in 2013. This was associated with emergence and predominance of genotype G12 RV, from 0% in 2011 to 66% in 2012 and 82% in 2013, infecting children from 1 month to 10 years of age. Two sequenced G12 strains, showed a Wa-like genome with genotype G12-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1, similar to the globally emerging G12 strains. Fecal anti-RV IgA analysis showed that most G12-infected and non-infected children had been in contact with either vaccine or wild RV strains, but such antibodies did not prevent symptomatic G12 infection. To conclude, in this study we have
Oral 43 - Impact of vaccination on rotavirus in the first year after introduction of the Rotarix® vaccine in England

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Keywords: England, Vaccine, Surveillance

Historically, in England rotavirus gastroenteritis has been associated with seasonal occurrence between January and March and responsible for an estimated 130,000 General Practitioner attendances and 13,000 hospitalisations per year, among children under 5 years of age. On the 1st July 2013 the Rotarix® rotavirus vaccine was added to the infant immunisation programme in England, and here we report on the impact of introduction of the vaccine in England in the first year from national surveillance data. In the 2013/14 season, laboratory-confirmed rotavirus infections were 67% lower than the ten-season average between 200/01-2012/13 for the same period. In 2013/14, the most substantial reduction was observed among 2-11 month olds (i.e. the age group targeted for immunisation). Laboratory strain surveillance shows a 48% reduction in the number of rotavirus-positive specimens referred for strain characterisation in January-June 2014 compared to the same period in 2013. An overall decrease in the numbers of wild-type-G1, G2, G4, G9 and G12 rotavirus strains has observed in the 2014 season compared with the previous season, whilst an increase in G3 strains and in vaccine-derived-G1 strains was observed.

Oral 44 - Changing pattern of rotavirus genotypes circulating in Australia since vaccine introduction

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Keywords: Genotypes, Emerging strains

INTRODUCTION:
Rotavirus is the major cause of gastroenteritis in young children worldwide, resulting in >450,000 deaths annually. In an effort to reduce the disease burden, two live oral rotavirus vaccines were developed; Rotarix® and RotaTeq®. Both vaccines were introduced into the Australian National Immunisation Program in July 2007. Each state and territory health agency has independently selected a rotavirus vaccine to include in its immunisation schedule. Studies from multiple Australian states have shown that emergency room visits and hospitalisation for rotavirus have declined dramatically since vaccine introduction. The simultaneous introduction of two rotavirus vaccines in Australian states and territories provides a unique opportunity to compare the impact of the different vaccines on the types of circulating rotavirus strains.

OBJECTIVE:
The aim of this study was to characterise the rotavirus genotypes circulating in Australia post vaccine introduction, and to compare the distribution of genotypes between states using different vaccines.

RESULTS:
Prior to vaccine introduction our long-term Australia-wide surveillance program has shown that G1P[8] strains were the dominant type identified in 7 of 11 years. Since vaccine introduction, the dominant type has changed every year, with G1P[8], G2P[4] and G3P[8] representing the most dominant types between 2007 and 2012. G12P[8] strains have emerged as an important genotype, being first identified in 2011, and becoming a significant cause of disease in Australian children during 2012, and the most prevalent genotype during 2013 and 2014. This represents the first time this genotype has been a major cause of disease in Australia. Australia is the only country where Rotarix™ and RotaTeq® are used in specific regions. Each year differences in genotype distribution were noted based on vaccine type. For
example, G2P[4] strains were more common in Rotarix™ locations during 2007, 2008 and 2012, but more common in RotaTeq® locations during 2009 and 2010. Rare genotypes and unusual genotype combinations have also been identified more frequently in the post vaccine era, with G3P[9] and G10P[14] strains emerging as a cause of disease in Australian children. Full genome analysis of G1P[8] and G2P[4] strains from the vaccine era suggests that these strains are genetically evolving, with changes occurring in outer capsid proteins.

CONCLUSION:
This data shows that the distribution of rotavirus genotypes is more diverse and dynamic since the introduction of the rotavirus vaccine program into Australia. Both rotavirus vaccines exert selective pressure on circulating strains, and in any given year the prevalent genotypes differ. However, when the entire post vaccine period is combined the overall genotype distribution appears to be similar.

Oral 45 - Host genetics and rotavirus infections – impact on epidemiology, immunology and vaccine take

NORDGREN Johan, BUCARDO Filemon, SHARMA Sumit, SVENSSON Lennart
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Keywords: Host genetics, Epidemiology, Vaccine

Recent in vitro studies have implicated human histo blood group antigens, specifically H-type I, Lewis b, H-type II precursor, and A antigen as receptors/ligands for rotavirus (RV) cell attachment in a genotype specific manner. In vivo studies from Burkina Faso, France, Nicaragua and Vietnam have further shown that P[8] infections occur predominantly in secretor positive children. Moreover, our ongoing studies have shown that secretor Swedish adults have higher RV specific IgG titers as well as higher neutralization titers to a G1P[8] strain than non-secretors, and that Nicaraguan non-secretor children have lower pre-vaccine anti-RV IgA titers in sera than secretors. Our study conducted in Burkina Faso and Nicaragua further showed that also the Lewis status was essential for RV susceptibility in genotype-dependent manner with P[8] infections only detected among Lewis b positive children and P[6] strains predominantly infected Lewis-negative children irrespective of their secretor status. These observations are important considering the higher percentage of Lewis-negatives in Africa and Latin America compared to Europe and North America. The results highlight the importance of host genetics in relation to molecular epidemiology and explain why P[6] RV are much more common in Africa than in Europe. The lower efficacy of P[8] based vaccines (Rotarix and Rotateq) in Africa could also be related to these host genetic factors. Here we address previous and ongoing studies regarding role of host genetics in RV infection with focus on epidemiology, immunology and vaccine take.

Oral 46 (short) & Poster 19 - Rotavirus genotypes circulating in Greece during the post vaccination era (2008-2014)

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Keywords: Rotavirus, Genotypes, Children

INTRODUCTION:
Rotavirus (RV) is the commonest cause of viral gastroenteritis in children. RV genotypes are classified according to 2 viral outer capsid proteins (VP7 and VP4) to G and P types respectively and present significant seasonal and geographical fluctuations. In 2007 two RV vaccines (monovalent-Rotarix and pentavalent-Rotateq) were licensed in Greece.

OBJECTIVES:
Describe the diversity of RV genotypes circulating in Greece and evaluate the impact of limited vaccine uptake (coverage 20-30%) in the natural fluctuation of the virus.

METHODS:

Faecal samples from children <5 years of age who visited emergency units of 19 Pediatric Departments with acute gastroenteritis between September 2008-August 2014. Samples were tested for RV Group A antigen with a rapid immunochromatography kit. Positive samples were further G and P typed through RT-PCR, multi nested PCR, gel electrophoresis and sequencing using specific primers for the VP7 and VP4 genes respectively.

RESULTS:

A total of 2286 samples were genotyped; male outnumbered female (55%). Mean age of children was 25 months old and 77% belonged to children ≤3 years old. Mean age of children was significantly raised in the season 2010/14 compared to 2008/10 (p<0.001). Seasonal peak of RV infection presented during the months January to March in all seasons except for the season 2008/09 and 2012/13 that was observed late in the spring (April to May). The most predominant types were G4P[8] (45%), G1P[8] (26%), G2P[4] (16.5%), G9P[8] (2.5%), G12P[8] (2.3%) and G3P[8] (2.2%). G4P[8] was the most predominant genotype in all seasons except 2010/11 and 2012/13 when G1P[8] was the most frequent. Significant increase in the detection of G2P[4] was observed in the seasons 2011/12, 2012/13 and 2013/14. Mixed and uncommon infections accounted for 3% and 2.5% respectively. Although genotypes were not associated with the gender or the age of the children, they differed geographically and temporally (p<0.001).

CONCLUSIONS:
The post-vaccine distribution of RV genotypes does not follow a specific pattern as a possible impact of RV vaccination in Greece. Continuous post-vaccine surveillance is essential for monitoring the current molecular epidemiology of RV and assessing the possible genetic evolution of RV strains as an effect of vaccine implementation.


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Keywords: Species A Rotavirus outbreak, G12P[8] genotype, RVA outbreak

INTRODUCTION:

Species A rotavirus (RVA) infection is the most common cause of severe gastroenteritis globally, with greater than 86% of deaths in low and middle-income countries. So far, 27 G and 37 P RVA genotypes have been described, but only a few combinations are commonly detected worldwide: G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8]. Genotype G12, previously considered atypical, are now being considered relatively common, as are being described in numerous papers around the world.

OBJECTIVES:
The aim of this study was to investigate the VP7 and VP8* sequences recovered from G12P[8] strains collected in a gastroenteritis outbreak observed in different Brazilian regions in 2014 to better understand the evolution of this genotype. In addition, we wanted to compare the antigenic regions of the outer capsid proteins of the Brazilian G12P[8] strains against G12 prototype strains and the RV1 RVA vaccine.

METHODS:

535 fecal samples collected between June and October 2014 from children with diarrhea disease in different Brazilian regions were analyzed. Genotypic and phylogenetic analysis were carried out, regarding VP4 (VP8*) and VP7 genes. Fifteen G12P[8] strains were elected to the VP8* and VP7 phylogenetic analysis.

RESULTS:

249 out of 535 received samples (46.5%) were positive for RVA with G12P[8] being detected in 214 (86.9%) of the RVA positive samples. G12 VP7 analysis revealed a high similarity between the Brazilian and African G12-III strains, especially one strain collected in Bhutan (BTN-120), one collected in Kenya (KDH651), one collected in Uganda (MRC-DPRU420) and two collected in South Africa (MRC-DPRU2140 and MRC-DPRU75). VP8* analysis showed that
all Brazilian G12-III strains belong to P[8]-3 lineage, the only P[8] lineage currently circulating in Brazil.

CONCLUSIONS:
No significant nucleotide/amino acid differences were detected between strains recovered from children and adults for both genes. The present study report a diarrhea outbreak in Brazil in 2014 associated with RVA genotype G12-IIIP[8]-3, similar to African strains. It is important to maintain the surveillance studies in order to identify possible changes in the epidemiological profile that can influence the effectiveness of the anti-RVA vaccination programs.

**Oral 48 (short) & Poster 21 - Rotavirus strains circulating in Finland - results from two years of national surveillance, 2013 and 2014, years 5 and 6 after introduction of rotavirus vaccination**


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2. Vaccine Research Center, University of Tampere, Finland

**Keywords**: gastroenteritis, National Surveillance, vaccine reassortant strain

**INTRODUCTION:**
Rotaviruses are the most common causative agents of children’s acute gastroenteritis in developed countries. Since 2009, Finland has included rotavirus vaccine (RotaTeq®) in the national immunization program (NIP) and since May 2013, rotaviruses have been included in the Finnish Communicable Diseases Act and Decree as part of the microbe strain collection. The Viral Infections Unit of National Institute for Health and Welfare (THL) has carried out rotavirus surveillance in the past two years in Finland in collaboration with the Vaccine Research Center, University of Tampere.

**OBJECTIVES:**
Before vaccination, rotavirus caused 2400 hospitalizations and 3600 outpatient clinic visits in children under 5 years of age per year. Upon vaccination rotavirus gastroenteritis has been reduced by more than 10 fold, however, the wild-type rotaviruses still circulate. The aim of the study was to characterize currently circulating rotavirus strains.

**METHODS:**
Diagnostic laboratories sent rotavirus positive specimens to THL’s Viral Infections Unit for typing. The samples were studied using either reverse transcription or real-time polymerase chain reaction (RT-qPCR) in THL’s Viral Infections Unit, or RT-PCR and sequencing in Vaccine Research Center.

**RESULTS:**
According to the National Infection Disease Register (NIDR), 282 and 274 rotavirus cases were registered during 2013 and 2014, respectively. Of these cases 71 (2013) and 176 (2014) samples were received in the Viral Infections Unit. Only 48% (2013) and 34% (2014) were collected from children under five years of age. The most common genotypes found were G1P[8] in 2013 (31%) and G2P[4] in 2014 (30%). Other frequently detected genotypes in both surveillance years were G4P[8], G3P[8], and G9P[8]. In addition genotypes G3P[9], G6P[14], G8P[8], G8P[14], G12P[8] and RotaTeq® vaccine reassortant strain were found rarely. Many of these less frequently detected genotypes occurred in adults.

**CONCLUSIONS:**
These two years represent years 5 and 6 since the introduction of RV vaccine into NIP in Finland. During these years, the genotypic distribution detected was not unusual. In children, the findings were similar to previous studies indicating that the vaccination with RotaTeq® has not effected on the rotavirus strains circulating in Finland.
Poster 01 - Characterization of the cell cycle arrest induced by rotavirus

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Keywords: cell cycle, kinesin, viroplasm

INTRODUCTION:
The rotavirus (RV) replication machinery requires a stabilized cytosolic microtubule-network and also the activity of kinesin Eg5. At the onset of mitosis and interphase the MT-network gets depolymerized, allowing the nucleation of short microtubules at the centrosomes, following the spindle assembly. We hypothesize that such rearrangements are detrimental for RV replication, making interference with MT-break-down essential, which in turn arrests the cell cycle prior to mitosis.

OBJECTIVES:
Demonstration of RV-induced cell cycle arrest and elucidation of the mechanism, by which RV achieves the MT-stabilization that lead to the cell cycle arrest.

METHODS:
We used synchronized RV-permissive cell lines, at the onset of the S-phase and monitored the cell cycle progression after infection with RV by staining with propidium iodide, followed by flow cytometry.

RESULTS:
Compared to non-infected cells, different RV strains, like the simians SA11 and RRV as well as the porcine OSU, were able to arrest infected cells in the S/G2 phase. In addition, we found that cell lines CV-1, Caco-2 and MDCK were also arrested in S/G2 phase upon RV infection. Interestingly, when RV was inactivated with UV-psoralen, which allows internalization of transcriptionally negative virions, the cell cycle arrest could not be observed. Additionally, we found that Eg5, which normally localizes near centrosomes, was re-located in the cytosol surrounding the RV-viroplasms in infected cells. Finally, we show a series of experiments aimed at elucidating the responsible viral component involved in the cell cycle arrest and in the re-distribution of the molecular motor surrounding the viroplasms.

CONCLUSIONS:
Our results highly suggest that RV uses a common pathway for the arrest of the host cell cycle that is RV strain and cell-line independent. RV impedes MT breakdown by arresting the cell cycle prior to mitosis (S/G2 phase), through a mechanism that is common between different RV strains and permissive cell lines. In addition, this mechanism requires viral transcription, suggesting a role of early expressed RV proteins. The re-localization of Eg5 upon RV infection, suggests a subversion of this host component that results in MT-stabilization in the interphase.

Poster 02 - Immune responses and protection in mice induced by parenteral and mucosal delivery of VP6 subunit rotavirus vaccine

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Keywords: VP6, intramuscular, intranasal

BACKGROUND:
Rotavirus (RV) is a common cause of severe gastroenteritis in infants and young children worldwide with high mortality in the developing world. Live oral RV vaccines are already part of routine childhood immunization, but these vaccines have shown lower efficacy in developing countries and are associated with safety concerns such as a risk of intussusception. We have developed a non-live subunit vaccine against RV in combination with norovirus (NoV), another major viral cause of acute gastroenteritis in children.
OBJECTIVES:
For protection against childhood gastroenteritis, we have introduced a concept of vaccination against RV and NoV with a combined trivalent vaccine consisting of RV rVP6 (subgroup SGII) protein and NoV GI-3 and GII-4 virus-like particles (VLPs). This study compares mucosal and parenteral immunization for induction of VP6-specific protective immunity in mice. For the RV VP6 component both rVP6 tubular structures and dl2/6-VLPs were evaluated.

METHODS:
BALB/c mice were immunized intramuscularly (IM) or intranasally (IN) with the candidate vaccine containing RV rVP6 protein or dl2/6-VLPs, and B- and T-cell immune responses induced by different delivery routes were measured. To assess protective efficacy, three weeks after the last immunization half of the mice were challenged orally with murine RV strain EDIMwt (non SGI/SGII, G3P[16]) and reduction in fecal RV antigen shedding was determined. Moreover, an ELISA-based antigen reduction neutralization assay was used to determine in vitro neutralizing activity of specimens of immunized mice against human Wa RV (SGII, G1P1A[8]) and rhesus RV (SGI, G3P5B[3]).

RESULTS:
Both vaccine formulations induced high levels of systemic cross-reactive serum IgG antibodies to VP6 as well as T cells. Mucosal IgG and IgA antibodies were induced by both immunization routes, although the IgA levels were considerably higher in IN immunized mice. Only IN route elicited detectable serum IgA responses. Mucosal anti-VP6 IgA antibodies inhibited replication of both RV strains in vitro. Most importantly, after the RV challenge ≥64% reduction in viral shedding was detected in IM and IN immunized groups. Increases in serum IgA responses were detected in all immunized mice after the challenge.

CONCLUSIONS:
Immunization with the trivalent vaccine containing either rVP6 or dl2/6-VLPs induced substantial protection in vitro and in vivo independently from the delivery route. These results underline the importance of non-serotype-specific protective immunity induced with the conserved VP6 protein.

Poster 03 - Screening for antibodies and their cognate epitopes that can block rotavirus-host cell interaction in the porcine model

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Keywords: Porcine, Antibodies, Epitopes

INTRODUCTION:
Rotavirus is an infectious disease that causes gastroenteritis in many animals including livestock such as pigs. Outbreaks in pig herds cause poor growth performance, an increase in morbidity and a higher mortality rate. This leads to a reduction in earnings for farmers, reduced sustainability and a lower quality of pork worldwide. Studies indicate that mammals produce antibodies against the VP4, VP6 and VP7 proteins of rotavirus during natural infections. We are screening serum from sows to identify antibodies that block the interaction between the porcine OSU strain of rotavirus with its host cell. Subsequently, we aim to screen potential epitopes of VP4, VP6 and VP7 to identify peptides that are recognised by antibodies that block rotavirus-host cell interaction.

OBJECTIVES:
Determine epitopes of three rotavirus capsid proteins that are recognised by antibodies that block rotavirus-host cell interaction. This work could lead to successful treatments against rotavirus in the porcine model.

METHODS:
Using bioinformatic techniques potential epitopes of VP4, VP6 and VP7 viral proteins that are exposed to the environment, have been generated from the protein sequence of OSU. These epitopes are predicted to be presented by the major histocompatibility complex-2 of the BALB/c mouse. ELISA and plaque reduction neutralisation assay are being used to screen for antibodies from the serum of sows, which recognise rotavirus and block its interaction with host cells. Following this, epitopes involved in the antibody-blocking of virus-host cell interaction are being identified by screening the ability of synthetic peptides to alleviate this
Poster 04 - Epidemiological and phylogenetic analysis of avian rotaviruses in Italy

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Keywords: Avian, phylogenetic analysis

INTRODUCTION:
Avian rotavirus (AvRV) causes enteritis of variable severity in young birds inducing severe economic losses to poultry industry. RVs are classified into eight groups (A-H) but only groups A, D, F and G were reported in avian species. Monitoring rotavirus distribution in both poultry (chickens and turkeys) and game birds is crucial to uncover strains diversity and to understand AvRV epidemiology in the field. Several studies on RVs occurrence and epidemiology in different avian species were reported worldwide. However, very little is known about the characterization of avian RVs in Italy.

OBJECTIVES:
The aim of this study was to provide information on: 1) distribution of the different RV groups in avian species suffering enteritis in Italy; 2) genetic diversity of RVs in these species; 3) dynamics and timing of RV infection within turkey flocks.

METHODOLOGY:
To study the distribution of RV groups, we analysed a total of 117 intestinal contents and/or faecal samples collected during the period 2006-2012 from birds of different species (76 chicken; 21 turkey; 10 pheasant; 5 guinea fowl; 5 partridge) all suffering enteritis and resulted positive for rotavirus by electron microscopy. To study the dynamics of infection, a longitudinal study was performed in 4 turkey flocks. Samples were weekly collected from grounding until 27-42 days old. Extracted viral RNA was subjected to RT-PCR assays with specific primer pairs for NSP4, VP6, VP4, VP7 of RV-A and RV-D groups and VP6 of RV-G and RV-F groups using the OneStep-RT-PCR kit (Qiagen). Nucleotide sequences were obtained using the same primers. Sequence alignment was performed using the CLUSTAL W method and phylogenetic trees were constructed using neighbour-joining method.

RESULTS:
One hundred and seven samples out of 117 (91.4%) were positive for group D AvRVs, 70 (59.8%) for group A, 61 (52.1%) for group F and 31 (26.5%) for group G. Single infections were present in 20 samples (17%) and multiple infections were present in 97 samples (83%) with different patterns. A group of 36 positive samples, representative by year and species, were selected for sequence analysis. Phylogenetic trees for VP4, VP7, NSP4 and VP6 segments were constructed on the basis of 115 complete nucleotide sequences. No correlation between year of isolation or avian species and the different RV-groups were observed in all the analysed segments. In some cases, segments of the same sample clustered in different clades evidencing gene reassortment events. Phylogenetic analysis of samples collected during the longitudinal study from 4 turkey flocks showed that different RV-groups and different strains from the same group were present in the same flock. However, such viral pattern changed along the study period within the farm.
CONCLUSIONS:
This study provides novel data on RVs prevalence in avian species in Italy showing both high presence of RV-A, D, F, G groups and great genetic variability. Longitudinal study in turkey flocks evidenced complex RV groups/strains patterns which change over time. Overall, these findings give the basis for further genomic and epidemiological studies aimed to better understand the characteristics of AvRvs circulating in Italy.

Poster 05 - Partial genomic analyses of Moroccan caprine rotavirus strains provide evidence for interspecies transmission

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Keywords: Caprine Rotavirus, Interspecies transmission, Nomadic lifestyle

The genome of group A rotaviruses (RAV) consists of 11 segments of double-stranded RNA. Of these, seg-4 and seg-9 encoded VP4 and VP7 are the major serotype determining proteins P-serotype and G-serotype respectively. Up to date, 27 G types and 35 P types are found worldwide. A co-circulation of different RVA serotypes may provide an opportunity to RVA strains to simultaneously infect the same animal species and/or humans with the potential for generation of reassortant viruses. The Bedouin livestock farming systems might favor the introduction of new strains from a heterologous host by interspecies transmission given their nomadic lifestyle. This preliminary study was carried out to provide some insights into the circulation of RVA in nomadic goats (19 kids (Chèv 1-19) and 5 ewes (Chèv 24) during a severe outbreak of diarrhea in April 2012 in Bouarfa, Eastern part of Morocco. These animals were raring with camels, sheep and cattle. RVA infection was determined with LSI VetMAX™ Triplex Ruminant Rotavirus & Coronavirus Real-Time PCR kit and the frequency of 40% was obtained. All positive samples were subjected to genotyping. RVA G10P[14] was detected in 56% of kids (Chèv 3, 4, 7, 8, 20) and G6P[14] genotype was found in one ewe (Chèv 21). Three cases (Chèv 1, 2, 22) have at least one G and/or P RVA untypable strains representing a portion of 33%. We carried out length sequencing of the genes encoding VP7 and VP 4 of two (Chèv 8 and Chèv 21) and eight (Chèv 1, 3, 4, 7, 8, 20, 21, 22) isolates respectively. Detailed molecular analysis revealed that VP7 sequence of the G10 caprine Moroccan isolate (RVA/Goat-wt/Mor/Chèv2/2012) showed maximum nucleotide similarity of 95% with the bovine strain B75/G10 isolated in India while the G6 Moroccan RVA/Goat-wt/Mor/Chèv21/2012/ presents a similarity of 91% with the human G6 isolate (RVA/Human-wt/BEL/B10925/1997). Whereas sequence analysis of the gene encoding the VP4 of all strains determine their genetic relation to the guanaco (Lama guanicoe) RVA/Guanaco-wt/ARG/Chubut/1999/G8P[14] (96% of nucleotide similarity) and to a less extend (92% of nucleotide similarity) with the RVA/Vicufa-wt/ARG/C75/2010/G8P[14]. Our study demonstrates that G10 serotype was common to nomadic goat kids, suggesting a high frequency of RVA transmission between goats and cattle. In addition, the G6 type was also detected and, thus transmission between this species and human is probable. Our study reveal for the first time that Moroccan caprine P[14] rotavirus strains can be the result of interspecies transmissions from camels.

Poster 06 - Circulation of pig group A and C rotaviruses in Belgian diarrheic suckling pigs and its impact on veterinary diagnostical analyses

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Keywords: rotavirus A, rotavirus C, suckling pigs

INTRODUCTION:
Group A and C rotaviruses have been identified as important causes of diarrhea in suckling piglets, together with Escherichia coli, Clostridium perfringens and Isospora suis. Though, the importance of rotavirus infections on Belgian pig farms has not been investigated, which
hampers the development of good strategies to diagnose and prevent this economical important disease.

OBJECTIVES:
In this study, it was aimed to investigate the presence of rotavirus A (RVA) and C (RVC) infections among Belgian diarrheic suckling pigs, in order to optimize current diagnostic strategies used in veterinary practice. Furthermore, the VP7 and VP4 genes of circulating strains were characterized, as this may benefit future vaccine formulation.

MATERIALS AND METHODS:
The presence of RVA, Escherichia coli, Clostridium perfringens and Isospora suis in diarrheic fecal samples (n=45) of sucking pigs less than 2 weeks old from 36 farms were investigated at a private diagnostic laboratory. However, veterinarians specified for which pathogens diagnostic tests should be performed. Here, RVA was diagnosed using a fast antigen detection strip, whereas bacteria were isolated on specific agars. Coccidia were purified using flottation, and visualised under a microscope. At the Laboratory of Virology, all samples were analyzed for RVA and RVC using RT-qPCR, and the genes encoding outer capsid proteins VP7 and VP4 were characterized by partial sequencing and phylogenetic analyses.

RESULTS:
Many of the common agents involved in the pathogenesis of diarrhea in sucking piglets were not routinely investigated in veterinary practice. However, in 61% of 36 farms tested, high viral loads of RVA (6.96 to 11.95 log10 copies/g feces) and/or RVC (5.40 to 11.63 log10 copies/g feces) could be detected, whereas rotavirus infections could only be diagnosed on 25% of the farms using a fast RVA antigen strip. Seventeen of these RVA strains were characterized, resulting in the detection of 4 different G-genotypes (G3, G4, G5 and G9) and 4 different P-genotypes (P[6], P[7], P[13] and P[23]) in 8 different G/P combinations. VP7 genotypes G5 and G4, and VP4 genotype P[7] were encountered most frequently (29.4% each). All RVC strains belonged to genotype G6 (VP7), except for one strain possessing the G1 genotype. Moreover, VP4 genes of Belgian RVC strains were genetically highly heterogeneous. Escherichia coli was also frequently isolated in the present study, but unfortunately the characterization of virulence factors was not requested routinely, making it difficult to interpret diagnostic results. Furthermore, most Clostridium perfringens strains were isolated from rotavirus negative samples. I. suis was only detected in 2 out of 45 samples, and probably underdiagnosed due to a lack of requests for routine testing in veterinary practice.

CONCLUSIONS:
As a conclusion, routine testing for RVA and RVC using RT-qPCR in diarrheic feces of sucking pigs is advised, but also diagnostic investigations of other pathogens should be carried out more frequently in order to come to sound conclusions, and to install durable and efficient prophylactic measures on affected pig farms.

Poster 07 - Isolation, identification and virological characterization of bovine rotaviruses from dairy calves, Morocco

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Keywords: Dairy calves, G10P[11] Rotavirus, Morocco

Group A Rotaviruses (RVA) are known to infect most commonly 0-3-month-old pre-weaned dairy calves causing major losses in the dairy and beef production/industry worldwide. In Morocco, very little reports exist as regards to this infection. The present study enrolled during 2014, aimed at the determination of RVA infection in three Moroccan dairy sectors where all dams are immunized against RVA (ROTAVEC-CORONA). New detection and isolation methods for RVA, followed by characterization and identification of RVA strains are presented. In total, 212 clinical samples from neonates’ diarrhea calves were registered and tested for RVA infection. The proportion of RVA positive samples was relatively high (24-
28.5%) and RVA strains were successfully isolated. Their identity was confirmed by checking the RNA with LSI VetMAX™ Triplex Ruminant Rotavirus & Coronavirus Real-Time PCR kit, France. Ct values varied between 12.2 and 32.18 with infectious titers ranging from 5.32 to 8.16 log TCID50/ml. Genotyping using molecular techniques confirmed the circulation of G10P[11] serotype in all dairies. The results are discussed in relation to the strategy of RVA prevention in dairy calves.

Poster 08 - Monitoring of rotavirus genotypes in indigenous children of Brazilian Midwest in the vaccine era: footprints of animal genome

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Keywords: molecular epidemiology, rotavirus vaccine, genotyping

INTRODUCTION:
Annually group A rotavirus (RVA) gastroenteritis accounts for approximately 1/3 of the total diarrheal deaths worldwide, and vaccination is considered effective in reducing the consequences of RVA. Although world RVA currently surveillance data provide useful estimates of the disease burden, they usually exclude from the sampling frame certain groups of children. Indigenous population is one group that might require special consideration.

OBJECTIVES:
The aim of this study was to describe the results of G- and P-types from Brazilian native children ≤3 years after vaccine introduction. Furthermore, selected strains have been analyzed for the VP7, VP6, VP4 and NSP4 encoding genes in order to gain insight into genetic variability of Brazilian strains.

METHODS:
A total of 149 samples, collected during 2008-2012, were tested for RVA using ELISA and PAGE, following by RT-PCR and sequencing.

RESULTS:
RVA infection was detected in 8.7% of samples (13/149). Genotype distribution showed a different profile for each year: G2P[4] in 2008 (25%; 4/16), G8P[6] in 2009 (7.5%; 5/66), G2P[4] in 2010 (7.1%; 3/42), G3P[8] in 2011 (5%; 1/20), and none in 2012 (0%; 0/5). The changes in genotype were found to be accompanied by a significant reduction in the detection rate of RVA from 25% (4/16) in 2008 to 0% (0/5) in 2012. The phylogenetic analysis of the VP7 and VP4 genes grouped the Brazilian G2P[4] and G3P[8] strains within the lineages currently circulating in humans worldwide. However, the phylogenetic analysis of the VP6 and NSP4 from the Brazilian G2P[4] strains, and the VP7 and NSP4 from the Brazilian G3P[8] strains suggest a distant common ancestor with different animal strains. Brazilian G2P[4] VP6 strains shared moderately high nucleotide identities with bovine (90.3-94.2% nt; 98.4-99.2% aa) and caprine (94.9% nt; 98.7-99.2% aa) I2 strains. Modest nucleotide identity was observed between Brazilian G3P[8] NSP4 E1 strain and the porcine strain A34 (90.1% nt; 72% aa); and between Brazilian G2P[4] NSP4 E2 strains and the goat strain G034 (90.5-91% nt; 78.8-80.2% aa). Strain G3P[8] VP7 shares moderately nucleotide identities with two feline G3 strains: BA222 and Cat2 (90.2% nt; 71.3-71.7% aa). The G8P[6] samples were analyzed in a previous study, and were documented to be closely related to bovine (VP7 and VP6) and bat (VP4) RVA strains.

CONCLUSION:
Despite the use of a convenience sample and the relatively small sample size, this is a pioneer study in Brazil focusing to monitor the RVA genotypes and to conduct genetic analyzes among indigenous children after the introduction of Rotarix™ in 2006. These results do not describe the Brazilian indigenous children population as a whole; nevertheless the epidemiological and genetic information obtained is expected to provide an updated understanding of RVA genotypes circulating in the native infant population, and to formulate policies for the use of RVA vaccines in indigenous Brazilian people. Moreover, these results highlight the great diversity of human RVA strains circulating in Brazil, and an in-depth surveillance of human and animal RVA will lead to a better understanding of the complex dynamics of RVA evolution.
Poster 09 - Molecular characterization of group A rotavirus genotypes in Oman between 2009 and 2013

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Keywords: Rotavirus, genotyping, epidemiology

BACKGROUND:
Diarrhea is a significant cause of morbidity and mortality worldwide. Especially affected are infants and young children below five years of age. Globally, rotavirus remains a major cause of morbidity and mortality in developing countries. Worldwide approximately 500,000 children die annually to rotavirus induced diarrhea.

OBJECTIVES:
To carry out a Hospital-based study on group A rotavirus gastroenteritis in children under five years of age to estimate the disease burden, and determine the circulating genotypes in Oman between 2009 and 2013 and genotypes diversity.

METHODS:
A total number of 6034 stool samples from hospitalized children less than 5 years of age with moderate to severe diarrhea were collected from 12 regional hospitals geographically representing the whole of Oman. They were screened for the presence of human Group A rotavirus antigen and the positive samples were genotype characterized by RT-PCR and/or sequencing.

RESULTS:
From the 6034 stool specimens investigated, 2931, 48.5 % were group A rotavirus antigen positive by ELISA. Four hundred and fifty of the 2931 positive samples (50 per year) representative of all the regions and of good integrity were selected for further analysis to determine their genotypes. Predominant genotypes on the VP7 gene were G1, G2, G3, G4, G9 and G12 respectively. Mixed infections between G1 G2 and G3 were seen in 18 samples (9.9%). Predominant genotypes on the VP4 gene were P[8], P[4] and P[6] respectively. Predominant G and P type combinations determined were G1P[8] and G2P[4].

CONCLUSION:
Diarrhea induced by group A rotavirus accounts for more than 45% of all diarrhea cases in Oman. Because improved sanitation or better health services does not have a direct impact on the prevalence of rotavirus disease, and the rate of positive cases needing hospitalization remains high in Oman, the primary public health intervention should be vaccination. The study results justify the introduction of rotavirus vaccination in Oman that will be part of EPI programme.

Poster 10 - Phylogenetic analysis of VP4 and VP7 coding sequences of Mozambican rotavirus strains

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Keywords: Phylogenetic analysis, Mozambican RVA

INTRODUCTION:
Although rotavirus surveillance has been carried out for many years in South Africa and other African countries, this is not the case in Mozambique. Rotavirus surveillance was introduced in 2012 in Manhiça, a rural site, and in 2013 in Mavaland, an urban site. Prevalence higher than 40% was established for rotavirus infections in these regions. Binary typing of strains using PCR indicated predominantly G2 and G12 genotypes in combination with P[4] and P[6]. Consequently, rotavirus vaccines will be introduced in Mozambique in September 2015.

OBJECTIVES:
The study focused on the confirmation of genotyping results using Sanger sequencing and determining phylogenetic relationships for the VP4 and VP7 coding sequences.

METHODS:
Double-stranded RNA was successfully extracted from 18 stool samples received from Mozambique using the TRizol® reagent. Of the 18 samples, 14 originated from Mavaland...
(urban) and 4 from Manhiça. Beg9 and End9, targeting genome segment 9, and Con2 and Con3, targeting genome segment 4, were used for cDNA synthesis. These primers were also used to amplify the coding regions of VP4 and VP7 using Kapa HiFi DNA polymerase. The amplicons were subsequently subjected to Sanger sequencing. Genotypes were confirmed by BLAST analysis of the resulting sequence data. Phylogenetic relationships were inferred using the Neighbor-Joining method in MEGA5.

RESULTS:
The genotyping results as determined with binary PCR typing were confirmed for all the strains. Most of the Mozambican G12 genotyped strains grouped with South African G12 strains. G2 strains MOZ/ROTA-439, MOZ/ROTA-428, MOZ/ROTA-440 and MOZ/ROTA-448 were closely related (95% bootstrap confidence) to G2 strains originating from Zimbabwe. P[6] identified sequences for MOZ/ROTA-051, MOZ/ROTA-278, MOZ/ROTA-040 and MOZ/ROTA-277 grouped with a South African and an Indian strain (95% bootstrap confidence). Although MOZ/ROTA-439 and MOZ/ROTA-428 genome segment 4 sequences grouped closely (70% bootstrap confidence) with a Zambian P[4] strain, most of the P[4] identified sequences formed a separate cluster (100% bootstrap confidence) during phylogenetic analysis. Interestingly, ROTA-304 clustered for both G2 and P[4] analysis with RVA strains originating from porcine. Preliminary results also did not suggest different clustering for sequences derived from strains which originated from the rural (Manhiça) and urban (Mavalane) sites.

CONCLUSIONS:
Phylogenetic analysis indicated that the Mozambican RVA strains analysed in this study are related to RVA strains circulating in Southern Africa. Furthermore, evolutionary analysis of ROTA-304 genome segment 4 and genome segment 9 indicated a close association with porcine originating RVA strains. This could be an indication of possible interspecies transmission.

Poster 11 - Rotavirus genotypes during pre-vaccine period in Ouagadougou, Burkina Faso


LaBESTA/CRSBAN/University of Ouagadougou/Burkina Faso

Keywords: Rotavirus, genotypes, Vaccination

INTRODUCTION:
Group A rotaviruses are the leading cause of severe diarrheal diseases and dehydration of infants and young children throughout the world. World Health Organisation (WHO) recommends the use of the vaccine strategy in order to reduce both mortality and hospitalization. Burkina Faso introduced the pentavalent RotaTeq* vaccine into the national immunization program in October 2013.

OBJECTIVES:
To describe molecular epidemiology of circulating rotavirus strains before vaccine introduction in Burkina Faso and to assess the potential impact of vaccination on the distribution of rotavirus genotypes.

METHODS:
Between November 2011 and September 2012, stools samples from children under five years old with (263) or without (50) diarrhea disorders for control group were collected in three hospitals of Ouagadougou. Rotaviruses were detected using real-time RT-PCR and rotavirus positive samples were G and P genotyped using end-point multiplex RT-PCR assays.

RESULTS:
Rotavirus was detected in 167 (64%) samples from children with gastroenteritis and in 9 (18%) samples from children without gastroenteritis. A significant difference was observed in the presence of rotavirus when comparing symptomatic and asymptomatic populations (p<0.01; OR = 7.92; 95%CI: 3.69 – 17.01). Among the rotavirus positive samples, both G and P type specificities could be assigned to 55% (n=96), only P-type for 5% (n=9), only G type for 10% (n=17) and 31% were non-typeable (n=54). Of note, samples that failed to be G and P genotyped presented the highest Ct values, suggesting that a low viral load could explain the genotyping failure. Among the 122 samples successfully genotyped (even partially), rotavirus genotype G9 (46%), G6 (17%) and G1 (13%) predominated. Genotypes G3 (7%), G12 (6%) and
G2 (4%) were detected at lower frequencies. Regarding the P genotypes, P [8] (59%) predominated, followed by P[6] (25%) and P[4] (2%). Of note, one sample presented a coinfection with P[4] and P[8] genotypes. A wide variety of rotavirus combinations was observed during this study, with G9P[8] (29%), G6P[6] (13%) and G1P[8] (12%) predominating. With a prevalence of 6%, this study reported for the first time the detection of rotavirus strains G12P[8] in Burkina Faso.

CONCLUSION:
This pre-vaccination study indicates a high prevalence of rotavirus infections in Burkinabe children and a strong association of rotavirus infections with illness. This study highlights the high diversity of rotavirus strains and confirms the high prevalence of G9P[8] and G6P[6] genotypes as previously described in children in Burkina Faso. These data constitute a baseline against which changes in circulating strains could be monitored after vaccine introduction.

**Poster 12 - Surveillance of rotavirus strains in Valencia, Spain, during 12 years (2003-2014)**

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**Keywords:** Rotavirus, surveillance, epidemiology

**INTRODUCTION:**
Group A rotavirus (RVA) is the leading cause of severe diarrhoea among infants and small children worldwide. Our laboratory contributes to monitor the distribution of rotavirus genotypes in Spain, collaborating with the European Rotavirus Surveillance Network (EuroRotaNet). Objective: To analyse the evolution of RVA genotypes along the years, before and after vaccine implementation.

**METHODS:**
Rotavirus infection was diagnosed by immunochromatographic (Certest Biotec) or ELISA (Meridian Bioscience, Inc.) tests used in the daily routine of our hospital. G and P genotypes were characterized in a total of 1,470 strains. RNA was extracted from 10% fecal suspensions by the guanidinium isothiocyanate/silica or by using Trizol reagent (Life Technologies). RVA genotyping was performed by multiplex RT-PCR followed by agarose gel electrophoresis, according to the standard procedures of the EuroRotaNet project (http://www.eurorota.net).

**RESULTS:**
As a whole, the distribution of G/P types along 12 years have been: G1P[8] 53.2%, G9P[8] 11.5%, G2P[4] 8.3%, G3P[8] 5.6%, G4P[8] 2.1%, G12P[8] 6.9%. Mixed infections with two or more genotypes were observed in 5.9% of the samples. G1P[8] was the predominant genotype in seven rotavirus seasons, in 2003-04, 2004-05, and during five consecutive seasons from 2007 to 2012. A sudden emergence of G9P[8] occurred in 2004-05, becoming G9 the predominant genotype in 2005-06 (84.6%) and in 2006-07 (68.3%). After being almost absent, G9P[8] re-emerged in 2010-11, representing 37% of all strains in 2012-13 and 45.4% in 2013-14. G12P[8] was detected in Valencia in the 2011-12 epidemic season and represented 29.6% of all typed strains in 2013-14. After introduction of rotavirus vaccines in 2007, a temporary increase of G2P[4] was observed in 2008-09 (18.8%) and in 2009-10 (20.7%). Uncommon RVA genotypes detected throughout the consecutive seasons have been G8P[6] (representing 5.3% of typed strains in 2008-09), G6P[14] (1 strain in 2007-08), G8P[14] (1 strain in 2009-10), G8P[4] (3 strains in 2010-11), and G3P[14] (1 strain in 2010-11). Whereas G12 strains belong to genogroup 1 (Wa-like), all G8 strains belong to genogroup 2 (DS-1-like).

**CONCLUSIONS:**
Rotavirus surveillance clearly shows the fluctuation of genotypes from season to season. Some emerging genotypes can persist circulating for years while others disappear in a short time.
**Poster 13 - The Italian Rotanet surveillance program. Rotavirus genotypes among children hospitalized with severe gastroenteritis, 2007-2014**

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**Keywords:** Surveillance, Rotavirus

**INTRODUCTION:**
Rotavirus is the major cause of acute gastroenteritis in infants worldwide. Despite most of the 450,000 annual deaths worldwide occur in developing countries, morbidity is high also in industrialized countries, calling for universal use of vaccines. In Italy, the Istituto Superiore di Sanità of Rome (ISS) has implemented a nationwide laboratory-based surveillance of acute rotavirus gastroenteritis, to investigate the diversity of rotavirus strains circulating before the introduction of large-scaled vaccination. RotaNet-Italy is linked to the EuroRotaNet network, including 17 European diagnostic laboratories.

**OBJECTIVES:**
This study was aimed to investigate the circulation of different rotavirus genotypes in Italy, describing geographic and temporal variations in the predominant viral types, and to detect the possible emergence of uncommon rotaviruses of animal or exotic origin.

**METHODS:**
From January 2007 to August 2014, approximately 9330 rotavirus positive stool samples were collected from pediatric patients with acute diarrhea hospitalized in 14 Regions throughout the Italian territory. After viral RNA extraction, samples were genotyped for VP7 (G-type) and VP4 (P-type) genes by reverse transcription and multiplex-PCR, using type-specific primers, in accordance with the EuroRotaNet methods and algorithm.

**RESULTS:**
Significant variation in the frequency of different rotavirus genotypes was observed between different years and areas of Italy. Most strains belonged to genotypes G1-G4, and G9, associated with either P[8] or P[4], commonly found in humans worldwide. Overall, the most common rotavirus genotype detected during the seven rotavirus seasons was G1P[8] (51%), followed by G9P[8] (16%), G4P[8] (10%), G2P[4] (8%) and G3P[8] (3%). However, in at least 3% of cases, unusual or novel strains, such as G3P[19], G6P[6], G6P[9], G8P[4] and G12P[8], were also detected, suggesting either gene reassortment events between rotaviruses of different origin or importation of strains from other countries. In particular, during the surveillance 2012-13 the diffusion of the emerging G12P[8] rotavirus genotype was unexpectedly detected in the Central Italian region of Umbria (6%), and in different regions during the following season 2013-14 (7%). Mixed infections with two or more rotavirus strains were observed frequently (8% of patients). Further characterization of strains by partial sequence analysis was carried out to evaluate the genomic evolution of viruses. Most rotavirus infection occurred in children <2 years of age, but cases were also reported in older subjects, identifying risks of infection through contact with infected children and increased susceptibility of the elderly population to rotavirus.

**CONCLUSIONS:**
Data from seven-year RotaNet-Italy surveillance confirm the genetic diversity of rotaviruses circulating in Italy, and the existence of remarkable differences between Regions and years. Although overall data confirm the suitability of present vaccine formulations, novel strains are also shown to emerge occasionally in Italy, which may unveil possible reassortment and/or zoonotic transmission. Rotavirus surveillance is valuable to investigate the diversity of rotavirus strains circulating and to control possible emergence of novel strains.

Full list of members is present at: http://www.iss.it/criv/index.php?lang=1&id=363&tipo=9
Poster 14 - Intra-genotypic characterization of group A rotavirus strains circulating in Germany (2008-2013)

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Keywords: phylogenetic analysis, intra-genotypic characterization, strain distribution

INTRODUCTION:
Group A Rotavirus (RVA) infections are still the most frequent cause of acute gastroenteritis among infants in Germany, despite the availability of vaccines. However, the number of reported RVA infections in Germany has decreased from 80.000 in 2007/8 to 33.000 cases in 2013/4. The influence of vaccination on circulating RVA strains remains unclear. Seasonal and regional distribution of RVA strains has been very heterogeneous. Therefore, continuous comparison of sequencing data from circulating RVA strains is needed to distinguish seasonal or regional fluctuation bias from possible global trends that could have an influence on vaccine efficacy.

OBJECTIVES:
Sequencing and intra-genotypic analysis of circulating RVA strains to identify possible trends that are shared among samples from different regions or seasons.

METHODS:
In addition to G and P typing, a subset of samples with common RVA genotypes (G1, G2, G3, G4 and G9) collected in different regions of Germany was further characterized by sequencing and phylogenetic analysis of VP7, VP4 and NSP4. These data were analysed with respect to genotype distribution and intra-genotypic differences.

RESULTS:
During 6 consecutive seasons (2007/8 to 2012/13), G and P typing data from >3000 samples and sequencing data (VP7, VP4, NSP4) of >10% of these samples were retrieved. By phylogenetic analysis, specific intra-genotypic patterns with respect to seasonal and regional distribution of some RVA sub-lineages or strains were observed.

CONCLUSIONS:
Despite of a heterogeneous mixture of co-circulating strains, different distribution patterns could be found, that are useful to monitor trends and identify possible emerging strains or beneficial strain characteristics.


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Keywords: Evolution, Italy, G3P[6]

BACKGROUND:
Group A rotaviruses (RVA) are the leading cause of acute gastroenteritis (AGE) in young children, causing up to 450.000 deaths worldwide, mostly in developing countries. Most of RVA infections in humans across developed areas of the planet, are related to five major G/P combinations: G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8]. During the surveillance activity of RotaNet-Italy, three uncommon G3P[6] rotavirus A (RVA) strains, designated as RVA/Human-wt/ITA/NA01/2009/G3P[6], RVA/Human-wt/ITA/NA06/2009/G3P[6], and RVA/Human-wt/ITA/NA19/2009/G3P[6], were identified in stool specimens from children with diarrhea hospitalized in Southern Italy in 2009.

OBJECTIVES:
After PCR genotyping following EuroRotaNet protocols, the samples NA01, NA06 and NA19 showed the G3P[6] genotype. To characterize the three RVA strains further, sequencing of the eleven genomic segments was planned and performed to investigate the origin of these uncommon RVA. RVA strains with a P[6] P-genotype in association with several G-genotypes have been isolated frequently in Africa, and sporadically also in developed countries. P[6] RVA strains have been detected in both patients with gastroenteritis and asymptomatic children, and P[6] has been established as a major P-genotype among porcine RVAs.

METHODS:
G- and P- genotyping was performed by reverse transcription-nested polymerase chain reaction (RT-nPCR), using mixtures of primers for either gene 9 and 4. For sequence analysis, RT-PCR reactions included primers specific for each gene investigated, using a Tm of 50°C for all reactions. A 3 min elongation step was used to obtain VP1-4, VP7 and NSP2-5 amplicons, whereas for VP6 and NSP1 elongation was protracted for 6 min. Multiple sequence alignments and phylogenetic tree construction were performed with MEGA6, applying the Maximum-Likelihood (ML) method; the substitution model for each tree was obtained by ModelTest from MEGA6.

RESULTS:
NA01, NA06 and NA19 RVA strains were found to possess the unusual genotype constellation G3-P[6]-I2-R2-C2-M2-A2-N2-T2-E2-H2. This study reports the first detection of uncommon G3P[6] RVA strains in human patients in continental Italy. The phylogenetic analysis of the eleven genomic segments showed no evidence of zoonosis or inter-species reassortment, revealing for the NA strains DS-1 a genomic constellation previously associated to human cases in Africa and Europe. The analysis of the hypervariable regions of VP7 and VP4 (VP8*) revealed high amino acid identity between the NA G3P[6] RVA strains involved in this study.

CONCLUSIONS:
The comparison of the G3 RVA strains investigated in this study and other G3 RVAs characterized previously in Italy reveals that a large variety of G3 genomic variants have been reported throughout Italy, which may be partially related to the persisting massive immigration from across the Mediterranean sea. The detection of exotic RVA strains also in developed countries highlights the importance of surveillance activity on rotaviruses, similar to other imported and emerging pathogens, in order to prepare at and control public health threats.

Poster 16 - Transient emergence of G12 rotaviruses in French infants
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Keywords: diarrhea in infants, G12 rotavirus emergence, clinical characterization

Group A rotavirus (RVA) are the leading cause of acute gastroenteritis (AGE) in young children worldwide. To follow up vaccine introductions, dedicated surveillance networks have been set up for investigating virological and clinical features of rotavirus infections. From 2009 to 2013, RVA-positive stool samples were collected from 3688 children under 5 years old admitted to the paediatric emergency units of 13 French large public hospitals. The genotyping of 3434 rotaviruses showed that G1P[8] strains (64.4% [55.3-73.2]) were predominant. G3P[8] (9.5% [1.5-19.0]), G9P[8] (8.3% [4.6-11.3]) and G2P[4] (8.1% [4.2-17.3]) strains had very changing incidence depending on seasons and regions, whilst G4P[8] (3.1% [0.8-7.2]) strains were mostly circulating locally. G12P[8] (2.1% [0.4-4.3]) strains emerged during the last two seasons with a prevalence of 4.3%. Most strains were associated with P[8] (89.2% [77.0-93.9]). Overall, 33 possible zoonotic reassortants (1.0% [0.4-1.5]) were also detected, such as G6 (27.2%) and G8 (18.2%), and were often associated with P[6] (69.7%). Among them, 2 G8P[8] strains were detected showing active recombination of bovine G8 with human P[8] strains, a first plausible step to human adaptation. Analysis of the clinical records of a group of 624 hospitalized children showed no difference in clinical manifestations in relation to genotype. Similarly, severity scores from 286 children show no difference in severity in relation to genotype. Among them, infection was severe in 51.7% of cases, but significantly less severe in children under 6 month old (P<0.0001). The relative stability of RVA genotypes currently co-circulating and the large predominance of P[8] type strains may ensure vaccine effectiveness in France. Establishing RVA genotypes remains however a key issue in understanding of the mechanisms by which strains emerge or are maintained in the population. The surveillance of rotavirus infections during ongoing and future vaccination programs will continue to monitor the emergence of new reassortants that may not respond to current vaccines and to help optimizing appropriate vaccine strategy against rotavirus disease, the more so as all genotypes can cause severe infections in infants.
Poster 17 - One year survey of human rotavirus strains suggests the emergence of genotype G12 in Apulia region (South Italy)

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Keywords: G12, emerging strain, South Italy

INTRODUCTION:
Group A rotaviruses (RV) are the most important etiological agents of acute gastroenteritis in infants and young children worldwide. A large number of rare or regionally common strains have been identified during surveillance in anticipation of vaccines introduction including G5, G6, G8, G9, G10, and G12 genotypes and P[1], P[3], P[6], P[9], P[11], P[14], P[19], and P[25] genotypes. Many of the newly described genotypes are thought to be of animal origin, including G9 that emerged subsequently as globally common G9P[8] strains. An increase of G12 strains has recently been reported in several countries, which is currently recognized as a globally emerging rotavirus genotype.

OBJECTIVES:
In this study the emergence of rotavirus A genotype G12 is reported in children hospitalized with acute gastroenteritis in Apulia region (South Italy) during 2014. A preliminary analysis of the VP7 segment has been performed to investigate the origin of the strains.

METHODS:
We collected 626 fecal samples from children hospitalized at the Giovanni XXIII regional pediatric referral hospital in Bari from January to December 2014. The population is mainly urban and the region whether is generally mild and dry. After viral RNA extraction, the genomic rotavirus RNA segments were reverse-transcribe and amplified with specific forward and reverse primers for VP7 and VP4 region. For G- and P-type determination the multiplex genotyping PCR assay were utilized according to the WHO protocol. VP7 gene of G12 strains was subjected to sequencing.

RESULTS:
Rotavirus antigen was detected in 81 of 626 samples (13%). Forty rotavirus strains (49%) were genotyped using a multiplex PCR and according to the WHO protocol. G1, G2 and G4 were detected respectively in 55%, 10% and 10% of cases. Nine samples (2.25%) showed the presence of genotype G12. In particular, 6 showed a genotype G12P[8] and 3 strains showed a mixed genotype G12+G9P[8]. The preliminary analysis of VP7 gene from two strains which were identical each other evidenced a similarity rate of 99% with the strain RVA/Human-wt/BTN BTN-120/2010/G12P[8] isolated in Bhutan (South East Asia) in 2010.

CONCLUSIONS:
A significant increasing of genotype G12 is emerging starting from 2014. In the period 2009-2013 surveillance of Rotavirus strains in Apulia region evidenced only sporadic cases of G12. Preliminary analysis of VP7 suggests the importation of such strains. Further monitoring is necessary to assess the real burden of rare genotypes in Apulia where a universal vaccination against Rotavirus has not been set up and the vaccine is offered only at children <1 year before they attend nursery schools or with chronic degenerative diseases at risk for complications and hospitalization for severe Rotavirus diarrhea.

Poster 18 - Unexpected diffusion of G12P[8] rotavirus strains among young children in a small area of central Italy

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Keywords: Rotavirus, G12P[8], Outbreak

INTRODUCTION:
Group A rotaviruses are the most common cause of acute gastroenteritis, causing every year up to 450,000 deaths among 0-5 years old children, mostly in developing countries. In 2007, the Istituto Superiore di Sanità activated a national surveillance program for rotavirus acute gastroenteritis (RotaNet-Italy) in collaboration with the Ministry of Health. RotaNet-Italy investigates the diversity of rotavirus genotypes circulating in Italy and the possible
emergence of uncommon or novel genotypes not represented in current vaccines. As in other countries of Europe, also in Italy rotavirus gastroenteritis is mainly associated with the five common human genotypes G1-G4 and G9, P[8] and P[4], but emerging strains including G6, G8 and G12 are also reported sporadically.

OBJECTIVES:
This study reports the unexpected diffusion of the emerging G12P[8] rotavirus genotype, occurred in the Central Italian region of Umbria during the rotavirus surveillance season 2012-13, while previously they had been reported only in rare sporadic cases throughout the country. In particular, this genotype had not been detected in the Italian region of Umbria during the previous season 2011-12, and it was not detected in neighboring regions in 2012-13. The aim of this study is focused on the understanding of the evolution and the genotype distribution of emerging strains.

METHODS:
Fifty-two G12 strains were genotyped for VP7 and VP4 and subjected to phylogenetic analysis. Amino acid sequences of antigenic regions of Umbria G12 strains were compared with vaccine and other field strains. Result. During the surveillance 2012-13 in Umbria, G12P[8] RVA were detected in 75% of RVA-positive samples collected, followed by G1P[8] (9%), G3P[8] (6%), G2P[4] (6%) and G4P[8] (4%). All G12 strains belonged to lineage III, and were associated with P[8] type. Sequence analysis showed close nucleotide identity of both VP4 and VP7 genes among Umbria G12P[8] strains. The VP7 gene was also similar to other G12 strains circulating in different years and countries, except for the Spanish G12 strains that showed a lower correlation with the Umbria strains, and clustered separately in the phylogenetic tree. Maximum Likelihood phylogenetic analysis indicated a close relationship with other local and global P[8] strains with different G-types. Overall findings suggest the introduction and evolution of a G12 VP7 gene into the local Wa-like rotavirus population. Comparisons of VP8* and VP7 antigenic regions showed high conservation between amino acid sequences of Umbria G12P[8] and other global G12 strains, and revealed various substitutions in the main antigenic regions between Italian G12 and RotaTeq® and Rotarix™ vaccines strains.

CONCLUSIONS:
The sudden and unexpected emergence of G12P[8] rotavirus confirms that these strains have the potential to become a sixth common genotype across the world. Continuous rotavirus strain surveillance in different countries is important to obtain a better understanding of rotavirus genotype distribution and evolution, particularly after vaccine introduction.

Poster 19 - Rotavirus genotypes circulating in Greece during the post vaccination era (2008-14)

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Keywords: Rotavirus, Genotypes, Children

INTRODUCTION:
Rotavirus (RV) is the commonest cause of viral gastroenteritis in children. RV genotypes are classified according to 2 viral outer capsid proteins (VP7 and VP4) to G and P types respectively and present significant seasonal and geographical fluctuations. In 2007 two RV vaccines (monovalent-Rotarix and pentavalent-Rotateq) were licensed in Greece.

OBJECTIVES:
Describe the diversity of RV genotypes circulating in Greece and evaluate the impact of limited vaccine uptake (coverage 20-30%) in the natural fluctuation of the virus.

METHODS:
Faecal samples from children <5 years of age who visited emergency units of 19 Pediatric Departments with acute gastroenteritis between September 2008-August 2014. Samples
were tested for RV Group A antigen with a rapid immunochromatography kit. Positive samples were further G and P typed through RT-PCR, multi nested PCR, gel electrophoresis and sequencing using specific primers for the VP7 and VP4 genes respectively.

RESULTS:
A total of 2286 samples were genotyped; male outnumbered female (55%). Mean age of children was 25 months old and 77% belonged to children ≤3 years old. Mean age of children was significantly raised in the season 2010/14 compared to 2008/10 (p<0.001). Seasonal peak of RV infection presented during the months January to March in all seasons except for the season 2008/09 and 2012/13 that was observed late in the spring (April to May). The most predominant types were G4P[8] (45%), G1P[8] (26%), G2P[4] (16.5%), G9P[8] (2.5%), G12P[8] (2.3%) and G3P[8] (2.2%). G4P[8] was the most predominant genotype in all seasons except 2010/11 and 2012/13 when G1P[8] was the most frequent. Significant increase in the detection of G2P[4] was observed in the seasons 2011/12, 2012/13 and 2013/14. Mixed and uncommon infections accounted for 3% and 2.5% respectively. Although genotypes were not associated with the gender or the age of the children, they differed geographically and temporally (p<0.001).

CONCLUSIONS:
The post-vaccine distribution of RV genotypes does not follow a specific pattern as a possible impact of RV vaccination in Greece. Continuous post-vaccine surveillance is essential for monitoring the current molecular epidemiology of RV and assessing the possible genetic evolution of RV strains as an effect of vaccine implementation.

Poster 20 - G12P[8] species A rotavirus causing gastroenteritis outbreak in different Brazilian regions in 2014: VP7 and VP8* genetic characterization

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Keywords: Species A Rotavirus outbreak, G12P[8] genotype, RVA outbreak

INTRODUCTION:
Species A rotavirus ( RVA) infection is the most common cause of severe gastroenteritis globally, with greater than 86% of deaths in low and middle-income countries. So far, 27 G and 37 P RVA genotypes have been described, but only a few combinations are commonly detected worldwide: G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8]. Genotype G12, previously considered atypical, are now being considered relatively common, as are being described in numerous papers around the world.

OBJECTIVES:
The aim of this study was to investigate the VP7 and VP8* sequences recovered from G12P[8] strains collected in a gastroenteritis outbreak observed in different Brazilian regions in 2014 to better understand the evolution of this genotype. In addition, we wanted to compare the antigenic regions of the outer capsid proteins of the Brazilian G12P[8] strains against G12 prototype strains and the RV1 RVA vaccine.

METHODS:
535 fecal samples collected between June and October 2014 from children with diarrhea in different Brazilian regions were analyzed. Genotypic and phylogenetic analysis were carried out, regarding VP4 (VP8*) and VP7 genes. Fifteen G12P[8] strains were elected to the VP8* and VP7 phylogenetic analysis.

RESULTS:
249 out of 535 received samples (46.5%) were positive for RVA with G12P[8] being detected in 214 (86.9%) of the RVA positive samples. G12 VP7 analysis revealed a high similarity between the Brazilian and African G12-III strains, especially one strain collected in Bhutan (BTN-120), one collected in Kenya (KDH651), one collected in Uganda (MRC-DPRU420) and two collected in South Africa (MRC-DPRU2140 and MRC-DPRU75). VP8* analysis showed that all Brazilian G12-III strains belong to P[8]-3 lineage, the only P[8] lineage currently circulating in Brazil.

CONCLUSIONS:
No significant nucleotide/amino acid differences were detected between strains recovered from children and adults for both genes. The present study report a diarrhea outbreak in
Brazil in 2014 associated with RVA genotype G12-IIIP[8]-3, similar to African strains. It is important to maintain the surveillance studies in order to identify possible changes in the epidemiological profile that can influence the effectiveness of the anti-RVA vaccination programs.

**Poster 21 - Rotavirus strains circulating in Finland - results from two years of national surveillance, 2013 and 2014, years 5 and 6 after introduction of rotavirus vaccination**


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**Keywords**: gastroenteritis, National Surveillance, vaccine reassortant strain

**INTRODUCTION:**  
Rotaviruses are the most common causative agents of children’s acute gastroenteritis in developed countries. Since 2009, Finland has included rotavirus vaccine (RotaTeq®) in the national immunization program (NIP) and since May 2013, rotaviruses have been included in the Finnish Communicable Diseases Act and Decree as part of the microbe strain collection. The Viral Infections Unit of National Institute for Health and Welfare (THL) has carried out rotavirus surveillance in the past two years in Finland in collaboration with the Vaccine Research Center, University of Tampere.

**OBJECTIVES:**  
Before vaccination, rotavirus caused 2400 hospitalizations and 3600 outpatient clinic visits in children under 5 years of age per year. Upon vaccination rotavirus gastroenteritis has been reduced by more than 10 fold, however, the wild-type rotaviruses still circulate. The aim of the study was to characterize currently circulating rotavirus strains.

**METHODS:**  
Diagnostic laboratories sent rotavirus positive specimens to THL’s Viral Infections Unit for typing. The samples were studied using either reverse transcription or real-time polymerase chain reaction (RT-qPCR) in THL’s Viral Infections Unit, or RT-PCR and sequencing in Vaccine Research Center.

**RESULTS:**  
According to the National Infection Disease Register (NIDR), 282 and 274 rotavirus cases were registered during 2013 and 2014, respectively. Of these cases 71 (2013) and 176 (2014) samples were received in the Viral Infections Unit. Only 48% (2013) and 34% (2014) were collected from children under five years of age. The most common genotypes found were G1P[8] in 2013 (31%) and G2P[4] in 2014 (30%). Other frequently detected genotypes in both surveillance years were G4P[8], G3P[8], and G9P[8]. In addition genotypes G3P[9], G6P[14], G8P[8], G8P[14], G12P[8] and RotaTeq® vaccine reassortant strain were found rarely. Many of these less frequently detected genotypes occurred in adults.

**CONCLUSIONS:**  
These two years represent years 5 and 6 since the introduction of RV vaccine into NIP in Finland. During these years, the genotypic distribution detected was not unusual. In children, the findings were similar to previous studies indicating that the vaccination with RotaTeq® has not effected on the rotavirus strains circulating in Finland.

**Poster 22 - Clinical and molecular descriptions of rotavirus in Morocco 2 years after Rotarix® introduction**

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**Keywords**: Rotavirus, Prevention, Morocco

**INTRODUCTION:**  
Group Rotavirus (RVA) is the most common pathogen causing acute gastroenteritis (AGE) in severe children <5 years mostly in developing countries. In Morocco, the Public Health
Ministry has introduced the Rotarix (RV-1) vaccine in late 2010 in the national immunization program followed by theRotateq (RV-5) in May 2014. Generalization is expected to significantly reduce severe AGE forms.

OBJECTIVES:
The present study aimed at the clinical and molecular descriptions of RVA in Morocco 2 years after RV-1 introduction.

METHODS:
The survey was performed on 153 hospitalized children with AGE in pediatric divisions in three different settings in Morocco (Hospital El Farabi at Oujda, Military teaching Hospital Med V at Rabat and Military Hospital at Dakhla) between April 2011 and May 2013. The Vesikari clinical score without any modification was used to evaluate the clinical severity of RVA and the stool samples were screened for the presence of RVA by Real time RT-PCR at the Centre de Reference des Virus Entéries (CNR) de Dijon, France. RVA positive samples were systematically confirmed and characterized by genotyping and sequencing.

RESULTS:
The prevalence of RVA positive children was 23% with a mean clinical score of 14.2. The occurrence of vomiting was significantly higher in RVA diarrhea than in non-RVA diarrhea (p < 0.001). When children were stratified according to RVA vaccination status, vaccinated children at Farabi site were found to be severely affected with AGE (p<0.03). Of note, this population of children was younger and has a mean age of 11.23 months (6.57-21.15). In non-vaccinated children, RVA G1P[8] was not detected and G2P[4], G3P[8] genotypes were very rare and followed by G9P[8]. RVA untypable strains were found in 3 non vaccinated children. In the population of vaccinated children, two cases were found infected with G2P[4], one case with G3P[8], two cases with G9P[8] and 13 children have at least one G and/or P not typables representing the greatest proportion (76%) of infected children in this group.

CONCLUSIONS:
RV-1 is demonstrated to protects against RVA G1P[8] serotype and was responsible for reducing the frequency of this infection in infants since late 2010, while other genotypes remained in circulation. This results support the strategy that the Moroccan Health Ministry has taken when RV-5 replaced RV-1 in 2014.

Poster 23 - Predominance of G12 rotavirus strains in Barcelona, Venezuela, following the introduction of the vaccine

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Keywords: G12 rotavirus, diarrhea, Venezuela

INTRODUCTION:
Venezuela was among the first developing countries to introduce rotavirus vaccines into their national immunization schedules. However, according to WHO data, there were no substantial changes in the prevalence of rotavirus diarrhea between 2006 and 2010 (staying around 31%). The circulation of several rotavirus type combinations raises concern about the vaccine efficacy. G12 has been recognized as a globally emerging genotype causing severe childhood diarrhea.

OBJECTIVES:
The aim of this study was to assess the prevalence and genotypes of rotavirus circulating in Venezuela.

METHODS:
During a surveillance study of diarrhea, a total of 27 (18%) rotavirus strains were detected by molecular assays from 150 stool samples, collected from children less than 5 years old suffering diarrhea in Barcelona, Anzoátegui State (Venezuela), during 12 months between 2012 and 2013.

RESULTS:
G and P genotypes were identified by multiplex RT-PCR, showing that G12 predominated in combination with P[8] (59.3%), followed by G2P[4] (22.2%), G9P[8] (7.4%), G1P[4] (3.7%) and G2P[NT] (3.7%). Most of the rotavirus strains were obtained from children who had been vaccinated. The eleven gene segments of four Venezuelan G12 rotaviruses were amplified and sequenced, and compared them with the genome of other rotavirus by BLAST. The
phylogenetic analysis revealed a conserved Wa-like genomic constellation G12-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1 for all rotavirus strains studied, which were genetically related to globally circulating human G12P[8] rotaviruses in most genes. The Venezuelan G12 strains were very closely related to each other in all the 11 gene segments, indicating that they shared common ancestral strains. VP7 gene analysis showed that all the Venezuelan G12 strains were identical among themselves and they had the greatest homology with the European human strain Dijon-R4969/2011/G12P8 (99.3% identity at the nucleotide level), clustering into the lineage II of G12. By alignment of the deduced amino acid sequences, multiple substitutions were identified within the hypervariable regions of VP7, including the 7-1a and 7-2 neutralization domains (97G*P, 125V*S, 217M*E and 221N*A) respect to amino acid VP7 sequence of Rotarix™ vaccine strain, the most used in Venezuela. VP4 genes of Venezuelan G12 rotaviruses showed the highest similarity (>99.5%) also with the human strains BE0258 and clustered within the P[8]-III lineage. No significant change was showed within the neutralization domains in the deduced amino acid sequences of VP4.

CONCLUSIONS:
To our best knowledge, this is the first report on the whole genome-based characterization of G12 strains that have emerged in Venezuela. Circulation of G12 genotype has also been described in other Latin American countries, but not with a such high frequency, consistent with a global spread and increasing epidemiologic importance of the G12 strains in the Americas. Because of this genotype is not covered by the current vaccines available, the relative high frequency of G12 rotavirus detected in Barcelona in vaccinated children suggests that the presence of this G type in Venezuela and the rotavirus vaccine efficacy should be monitored carefully.

**Poster 24 - Evaluation of a Taqman Array Card test for group A rotavirus detection and genotyping in Brazilian stool samples**


*Fundacao Oswaldo Cruz, Rio de Janeiro, Brazil*

**Keywords**: Rotavirus, real-time PCR

**INTRODUCTION:**
Group A Rotaviruses (RVA) represent the main cause of acute gastroenteritis in children under five years old worldwide. A sensitive molecular technique is important to ensure reliable results for epidemiological surveys. The TaqMan Array Card (TAC) system is a 384-well singleplex real-time PCR format that allows detection of multiple infection targets. Here we used a TAC that has been developed for detection of 19 enteropathogens, including characterization of eight G genotypes (VP7 gene) and six P genotypes (VP4 gene) of RVA.

**OBJECTIVES:**
Evaluate the RVA detection and genotyping results obtained with the TAC method comparing with the previous results obtained by polyacrylamide gel electrophoresis (PAGE), enzyme immunoassay (EIA) (Ridascreen, R-Biopharm) and RT-PCR for RVA genotyping.

**METHODS:**
One-hundred and thirty-nine samples were processed by TAC out of a three-hundred total selection. All samples were previously tested by PAGE, EIA and RVA genotyping by RT-PCR. A modified extraction procedure was performed to isolate both DNA and RNA from stool samples using the QIAamp Fast DNA Stool Mini Kit. Extrinsic controls PhHV (Phocine Herpesvirus) and MS2 (bacteriophage) were added to samples during the lysate preparation to evaluate extraction and amplification efficiencies. TaqMan Array Cards were used to amplify nucleic acid. RVA detection and genotyping below a quantification cycle (Cq) of 35 and 40, respectively, were used as a cutoff for test positivity based on a limit of detection.

**RESULTS:**
Six samples were previously diagnosed as RVA positive by PAGE, EIA and genotyped by RT-PCR as G2P4 (n=3), G1P8 (n=1), G3P8 (n=1) and G2P8 (n=1). Our TAC preliminary results confirmed RVA detection in all six samples and genotyping by TAC confirmed G1P8, G3P8 and G2P4 (n=1) strains. The other G2P4 (n=2) strains were characterized as mixed infection with P8 and the G2P8 (n=1) strain was characterized as G12.

**CONCLUSION:**
The TAC method is a specific and rapid method for simultaneous detection of nucleic acids from viruses, bacteria, protozoa and helminthes, as well as genotyping RVA, in the same test. The specificity of the TAC method compared to RT-PCR for RVA genotyping is suitable, once one non-typed G sample was characterized. To date, one-hundred and thirty-nine samples were tested for RVA by TAC method yet, but a set of one-hundred and sixty-one samples will be processed to allow a more extensive evaluation of specificity and sensitivity of TAC results. 

Financial support: Bill and Melinda Gates Foundation, CDC and IOC-FIOCRUZ

Poster 25 - Diagnostic accuracy of seven commercial assays for the rapid detection of group A rotavirus antigens

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Keywords: Sensitivity and Specificity, Immunochromatography

INTRODUCTION:
Group A rotavirus (RVA) is the most common cause of viral gastroenteritis during childhood. Rapid tests as immunochromatographic (ICT) assays are widely used to detect RVA antigens in stool samples. Regular assessment of the diagnostic performances of ICT assays is an essential issue to accurately diagnose RVA infections.

OBJECTIVES:
The purpose of this study was to compare diagnostic accuracy between seven commercial ICT assays intended for the detection of RVA antigens in human stool samples.

METHODS:
All raw fecal samples with enough quantity (i.e. more than 1g of feces) and for which a rotavirus detection has been performed from February through May 2014 by three French hospitals were included in the study. Samples were included independently of the initial rotavirus result and whether the patient presented gastroenteritis symptoms or not. RVA diagnostic accuracy was assessed in parallel for seven commercial ICT assays using an in-house quantitative real-time RT-PCR assay as a reference.

RESULTS:
Sensitivity of the ICT assays ranged from 69.1% (95% CI: 59.6-77.6) to 78.2% (95% CI: 69.3-85.5) and the specificity ranged from 97.9% (95% CI: 94.0-99.6) to 100% (95% CI: 95.8-100). The positive likelihood ratios of the ICT assays were high and ranged from 36.8 (95% CI: 12.0-113.4) to 224.4 (95% CI: 14.1-3577.2). The negative likelihood ratios ranged from 0.221 (95% CI: 0.156-0.314) to 0.312 (95% CI: 0.236-0.412). Finally, high diagnostic odds ratios were observed, with values ranging from 158.7 (95% CI: 46.5-541.5) to 1013.3 (95% CI: 60.8-16875.1). It appeared that the ICT assays were unable to detect RVA at low viral loads as measured by the quantitative real-time RT-PCR assay, and that the low rotavirus load corresponded to asymptotically infected individuals. Considering only the symptomatically infected individuals, a potential increase of the sensitivity of the ICT assays was observed, with sensitivity ranging from 78.5% (95%CI: 67.8-86.9%) to 88.6% (95%CI: 79.5-94.7%).

CONCLUSIONS:
The RVA diagnostic accuracies of the seven ICT assays were similar. These assays are suitable for the rapid diagnosis of RVA in symptomatically infected individuals, but their inability to detect asymptotically infected individuals could raise concerns about the prevention of nosocomial infections.
Poster 26 - Retrospective assessment of Vikia® Rota-Adeno and premier Rotaclone® tests compared to reverse transcription polymerase chain reaction for detection of group A rotavirus

LAGARE Adamou
CERMES, Niamey, Niger

BACKGROUND:
Rotavirus is the most common cause of severe diarrhea in young children. Although enzyme immunoassays (EIA) are the recommended tests for detection of rotavirus in stool samples, rapid diagnostic tests (RDT) may be better suited for use in peripheral health centers or ambulatory services.

OBJECTIVES:
We conducted a parallel retrospective evaluation of the diagnostic accuracy of Vikia® Rota-Adeno test (RDT) and PremierTM Rotaclone® (EIA) assay using reverse transcription polymerase chain reaction (RT-PCR) as the reference standard. Study design: We randomly selected 119 RT-PCR positive and 132 RT-PCR negative samples out of 734 stool specimens previously tested with Vikia® Rota-Adeno and characterized by RT-PCR. Selected samples were tested with the Vikia® Rota-Adeno and the PremierTM Rotaclone® and read by two technicians blinded to the results.

RESULTS:
The sensitivity of both tests was 80.7%. After exclusion of one indeterminate result by visual reading, the specificity of the PremierTM Rotaclone® was 100% by visual or optical density readings and that of Vikia® Rota-Adeno test was 95.5%. Inter-reader agreement was excellent for both tests (kappa=1).

CONCLUSION:
Considering the better specificity of the EIA assay compared to the RDT, it should be preferred for surveillance and diagnostic purposes whenever laboratory capability allows. However, the similar sensitivity and acceptable specificity of the rapid test make it a good alternative for use in peripheral health centres.

Poster 27 - False-positive rotavirus results are not completely avoided by pre-RT-qPCR treatments with propidium monoazide or RNase

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Keywords: PMA treatment, RNase treatment, rotavirus RT-qPCR

INTRODUCTION:
Detection of viruses causing acute gastroenteritis (AGE) in humans relies mostly on (reverse transcription) - polymerase chain reaction ((RT)-PCR) due to its sensitivity, specificity and rapidity. (RT)-PCR does not, however, provide information on infectivity, and thus it may overestimate the importance of viral findings especially in environmental samples.

OBJECTIVES:
The aim of this study was to investigate the ability of propidium monoazide (PMA), an intercalating dye, to bind free rotavirus RNA and thus render it undetectable in RT-PCR. RNase treatment of both non-inactivated and heat-inactivated virus was included in order to evaluate its ability to eliminate free RNA originating from damaged virions.

METHODS:
Human rotavirus strain Wa propagated in MA-104 cells was used throughout the study. The diluted stock (3.3 x 10^3 TCID50/ml) was divided in two aliquots and the other one treated with RNase before further experiments. Incubation at 80 °C for different time periods (1 – 60 min) was used to inactivate the virus, and residual infectivity was confirmed at every time point by the TCID50 method. Infectious and inactivated samples were treated with either PMA or RNase. After extraction of viral RNA, the samples were subjected to RT-qPCR performed with rotavirus-specific primers and a probe.

RESULTS:
The infectivity of rotavirus Wa decreased rapidly after 1 min incubation at 80°C, and after 5 min it became undetectable by TCID50 assay. In RT-qPCR the viral titer remained relatively constant regardless of the inactivation time. Based on preliminary tests, unbound or excess
PMA was efficiently purified from the samples by nucleic acid extraction and therefore it did not have an inhibitory effect on RT-qPCR. A PMA concentration of 100 µM was chosen since it was able to decrease the RT-qPCR titer of purified rotavirus RNA by 3.0 log10. PMA treatment of the virus aliquot not preliminary treated with RNase significantly reduced the RT-qPCR titer of samples taken at time point 0, but the titer did not decrease further even when the virus was incubated at 80 °C for 60 min. RNase treatment after heat inactivation reduced the titer of samples taken at time point 0 by 0.8 log10, and after 5 min incubation at 80°C, the reduction in the titer was 2.6 log10. When the experiments were repeated with the virus aliquot subjected to RNase treatment prior to heat inactivation, PMA decreased the titer 0.5 – 1.8 log10 depending on the inactivation time. The effect of the additional RNase treatment was less profound (max 0.6 log10).

CONCLUSIONS:
In this study, complete inactivation of samples did not alter the RT-qPCR titer of samples treated with PMA or RNase as expected. However, both PMA and RNase decreased the level of detected virus, so pre-RT-qPCR treatments with these agents are promising alternatives when assessing the infectivity of viruses. Further validation of these methods, with less complex matrices and different inactivation procedures, are needed before they can be applied to environmental samples.

Poster 28 - Towards a validation of a serum neutralization test to control human rotavirus vaccine efficacies in Morocco

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Keywords: Serum neutralization test, Human rotavirus vaccine efficacy, Morocco

Acute gastroenteritis (AGE) is a serious cause of morbidity and mortality in children less than five years of age in developing countries and group A rotaviruses (RVA) are implicated as the major viral pathogens. Today, anti-RVA vaccines offer the best strategy for preventing severe AGE. World Health Organization pre-qualified two vaccines, the Rotarix (RV-1) and the RotaTeq (RV-5) as safe and effective in all regions of the world. In Morocco, RV-1 and RV-5 were introduced into the National Immunization Program since 2010 and 2014 respectively and RV-1 has been proven to have clinical efficacy. To improve the study of efficacy of both vaccines in Morocco, we have developed a standard Serumneutralisation test (SNT) to elicit titers of neutralizing activity to G1P[8], G2P[4], G3P[8], G2P[8] and G9P[8] human strains and IgG avidity in guinea pigs. The strains were isolated in MA104 cells and further adapted in Vero cells. They were identified using virological and molecular characterizations. Their infectious titers ranged from 5.8 to 7.8 log TCID50/ml. For each strain, neutralizing activity in Guinea pigs serum is titrated in the neutralization test against 100 TCID50/50µl of its homologous virus. Prior to incubation of virus and serum mixtures for 1 hour at 37°C, viruses were treated with trypsin at a concentration of 3 µg/inoculum at 37°C for 30 min. Trypsin was also added to the Vero cell suspension in maintenance media at a concentration of 1 µg/ml. This neutralization test will be carried out to monitor the epidemiological situation of RVA infection in children during pre and post vaccination era in Morocco (2001-2015).

Acknowledgements: The authors are grateful to Professor P.Pothier for supplying the MA104 cell line.
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