Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company’s public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
viral pathogens (50.0%), especially among adults (85.7%). In children, influenza viruses, RSV and rhinovirus (32.1%) were detected frequently (Table 1).

**Conclusion:** Respiratory viruses are significant causes of severe ARI in adults and young children during the winter season. Early recognition of viral pathogens in ARI etiology is important in order to diagnosis and management of severe ARI in ICUs.

http://dx.doi.org/10.1016/j.jcv.2016.08.251

**Abstract no:** 332  
**Presentation at ESCV 2016: Poster 212**

**Identification of mutations in surface glycoprotein genes of human respiratory syncytial virus in children treated with palivizumab**

A. Flammang 1,*, J. Hamel 1,2, J. Brouard 1,4, L. Adamon 2, A. Vabret 1,4, J. Dina 1,4

1 CHU de Caen, Department of Virology, Caen F-14000, France  
2 National Reference Center for Measles and Respiratory Paramyxoviruses, France  
3 CHU de Caen, Department of Pediatrics, Caen F-14000, France  
4 Université Caen Normandie, Medical School, Caen F-14000, France  
5 CHU de Caen, Department of Neonatology, Caen F-14000, France

**Background:** Palivizumab is a respiratory syncytial virus (RSV)-neutralizing monoclonal antibody clinically used for the prevention of severe RSV infections in high-risk infants, preterm infants and infants with hemodynamically significant heart disease or chronic lung disease. Palivizumab acts by blocking the fusion step of virus replication. Mutants resistant to palivizumab were isolated in vitro and also in children with RSV infection while receiving palivizumab. The mutations reported are situated on the fusion protein (amino acids 262–276), in the liaison site of the palivizumab. It seems that mutations out of the liaison site do not confer resistance to palivizumab. The gene coding for the attachment glycoprotein (G gene) was rarely sequenced. The aim of this study was to analyze the complete F and G genes sequences coding the surface glycoproteins of RSV isolates collected from patients receiving palivizumab.

**Material and methods:** RSV isolates were obtained from nasopharyngeal swabs of high-risk infants treated with palivizumab at the University Hospital of Caen between October 2011 and April 2016 and having presented a RSV-breakthrough during the treatment or in the six months after. RSV controls were obtained from infants who did not receive palivizumab. Viral ARN was extracted using Qiasymphony DSP Virus/Pathogen Mini kit®. The group typing of hRSV. A or B, was completed using real time-RT-PCR. The amplification and sequencing of the complete F and G genes were performed using One-Step RT-PCR kit® (Qiagen, Hilden, Germany) and specific primers and protocols. The analysis and comparison of the obtained sequences with reference strains and control sequences were performed with BioEdit® software. Phylogenetic tree were constructed by the neighbor-joining method in MEGA 6.0® software.

**Results:** Among the 273 infants treated with palivizumab during the period of the study, 15 (8.4%) have presented a RSV infection during their treatment or in the six months after. Seven RSV/A and 8 RSV/B were identified by real-time PCR. The amplification and sequencing of the F and G genes were successfully undertaken.

For the RSV/A analysis, phylogenetic trees were constructed using 6 RSV/A detected, one control RSV/A and 42 reference sequences. The hRSV/A isolated in 2014 or after were identified in the ON1 cluster. When they were detected in 2011 they clustered with the GA2 genotype. None RSV/A was detected between 2011 and 2014.

The analysis of complete F genes alignments of hRSV/A shows several mutations out of the liaison site of palivizumab. We found one mutation in the liaison site, the N276S mutation. This was previously described as a mutation conferring partial resistance to palivizumab in *vivo* and *in vitro*. This mutation was also identified in the viruses collected from the control population.

**Conclusion:** This study allowed us to characterize mutations of RSV in case of palivizumab treatment failure.

http://dx.doi.org/10.1016/j.jcv.2016.08.252

**Abstract no:** 340  
**Presentation at ESCV 2016: Poster 213**

**The use of in vitro human airway epithelia for the development of novel antivirals**

S. Huang 1,*, S. Benaudia 1, R. Bonfante 1, B. Boda 1, M. Essaidi-Lazioni 2, L. Wisnewski 1, L. Kaiser 2, C. Tapparel 2, S. Constant 1

1 Epithelix, Geneva, Switzerland  
2 Laboratory of Virology, University of Geneva Hospitals, Switzerland

The human airway epithelium occupy a central position in the pathogenesis of respiratory viruses. As the first line of defense against microorganisms, epithelia cells react through mucus secretion, mucociliary clearance, activation and release of chemokines, cytokines, lipids, growth factors, proteases, etc. Viral respiratory infections are the most frequent etiologies of acute illnesses worldwide and cause mild to severe diseases such as common cold, bronchiolitis and pneumonia. A comparative study was carried out on the infectivity and replication of the most frequent human respiratory viruses using standardized in vitro
reconstituted human airway epithelia (MucilAir™). Differentiated tissues were infected in parallel with clinically relevant strains of rhinovirus (A16, A49, A55, B48, C8, C15), respiratory enterovirus (EV68), influenza virus (H3N2) and corona virus (OC43). For each virus, replication kinetics, cell tropism, impact of the virus on tissue integrity and cilia function were assessed.

Development and use of anti-viral drugs are one of the priorities for major pharmaceutical companies. As proof-of-concept for drug screening, the efficacy of Rupintruvir and Oseltamivir were tested in MucilAir™. Rupintruvir efficiently inhibited the replication of HRV-A16 and HRV-C15 in a dose and time dependent manner (up to 99% inhibition). Interestingly, (i) Oseltamivir reduced the replication of H1N1 and H3N2 and restored the impaired barrier function monitored by Trans-Epithelial Electrical Resistance and (ii) Rupintruvir restored the mucociliary clearance impaired by EV68 (7 μm/s for the Mock up to 40 μm/s for the Rupintruvir treatment at 50 nM at 96 h post inoculation).

These results demonstrated that MucilAir™ is a robust, reliable and relevant tool for antiviral drug development.

http://dx.doi.org/10.1016/j.jcv.2016.08.253

Abstract no: 346
Presentation at ESCV 2016: Poster 214

Study on immunological characteristics of monoclonal antibodies produced against the Kazakhstan isolates of influenza A(H1N1) virus

N.G. Klivlevyeva∗, T.I. Glebova, M.G. Shamenova
Institute of Microbiology and Virology, Kazakhstan

The main economic and social damage resulting from infectious diseases throughout the world is caused by acute respiratory viral infections and influenza. In recent years, the epidemic process is characterized by co-circulation of influenza virus subtypes A(H1N1), A(H3N2) and type B. In determining the etiology of viral infection serological analysis is one of the fundamental components. Monoclonal antibodies (MAbs) permit to dramatically increase the specificity and sensitivity of diagnostic techniques for the detection of viral antigens. Immunological characteristics of MAbs produced against the Kazakhstan isolates of influenza A(H1N1) virus were studied with immunofluorescence, HAI and microneutralization assays. Immunofluorescence testing revealed that MAbs are specific against homologous and related antigens, and identified them in the form of distinct granular fluorescence before the conjugate dilutions of 1:80–1:160. It was found that MAbs in HAI assay revealed a wide range of responses and in high titres (1:160–1:10240) inhibited the hemagglutinating activity of the homologous and related reference and Kazakhstan influenza viruses and did not react with the heterologous A(H3N2) and type B viruses. In microneutralization assay MAbs neutralized influenza A(H1N1) viruses and did not react with influenza viruses A(H3N2) and type B. Thereby, the similar spectra of MAb reactivity against A/H1N1 viruses indicate the presence of antigenic determinants in the HA composition of all the investigated viruses, that allows to recommend the resulting MAbs for differentiation of A(H1N1) viruses from the seasonal A(H3N2) and type B strains.

http://dx.doi.org/10.1016/j.jcv.2016.08.254

Abstract no: 38
Presentation at ESCV 2016: Poster 215

Genetic diversity and characteristics of porcine reproductive and respiratory syndrome virus in the area of Korea from 2013 to 2015

I.O. Ouh, J.E. Yu, H. Kang, J. Lee, S.E. Choe, I.S. Cho, S.H. Cha∗

Viral Disease Division, Animal and Plant Quarantine Agency, Gimcheon 39660, Republic of Korea

Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is rapidly gaining importance as one of the most economically significant diseases in swine worldwide. PRRSV is an enveloped positive single-stranded RNA virus that can be divided into two different genotypes, the European genotype (type 1) and the North American genotype (type 2). The genome of PRRSV is approximately 15 kb in length and contains at least 20 open reading frames (ORFs). ORF5, encoding Gp5, is one of the most variable regions of the PRRSV genome, and often used to examine genetic diversity and monitor evolution of PRRSV. In this study, the recent isolates in the field were evaluated for genetic variation based on ORF5 nucleotide and amino acid sequence.

Materials and methods: Lung and serum samples were collected from 541 pig farms in nationwide where clinical symptoms had been observed in 2013–2015. Total RNA was extracted from serum and lung using an RNeasy mini Kit (QIAGEN) according to the manufacturer’s protocol. To obtain sequences of the complete ORF5, reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out using the One Step RT-PCR Kit (QIAGEN) and PRRSV primer sets derived from sequences of the ORF4-6 of the LV and VR-2332 strain, respectively. Multiple sequence alignments and phylogenetic trees were carried out using CLC Main Workbench 7.0.3 and Mega 6 program. Bootstrap values were calculated on 1000 replicates of the alignments to assess the confidence limits of the branching.

Results: To more totally understand the genetic diversity and characteristics of PRRSV in the area of Korea, we analyzed the open reading frame (ORF) 5 sequences of 323 (type 1) and 269 (type 2) of PRRSV from 2013 to 2015. The results showed that both types 1 and 2 have been circulating in Korea pig farms and that the regional rate of infection was more prevalent in Gyeongsangnam-do province in Korea. Type 1 PRRSVs from Korea are clustered in subtype 1, subgroup A, B, and C. Type 2 PRRSVs are classified in lineage 1, 4, 5 and new Korea subgroup A, B. Recently, the genetics of type 2 PRRSVs in Korea have become unique regional characteristics in Gyeongsangnam-do. Recently, the genetics of PRRSVs in Asia have become more diverse. Although the genetics of type 2 PRRSV in Korea have unique regional characteristics in Gyeongsangnam-do, the genetics of PRRSV in Asia have become more diverse.

Conclusions: This study of PRRSV in different geographical areas should be performed regularly to monitor field isolates. This would provide annual genetic information for PRRS control and vaccine selection and/or renewal [1,2].

Reference
[1] E.J. Choi, et al., Genetic diversity of porcine reproductive and respiratory syndrome virus in Korea, J. Vet. Sci. 14 (2) (2013) 115–124.
[2] S.H. Kim, et al., A molecular analysis of European porcine reproductive and respiratory syndrome virus isolated in South Korea, Vet. Microbiol. 143 (2010) 394–400.

http://dx.doi.org/10.1016/j.jcv.2016.08.255