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**P.059****Detection of Epstein–Barr Virus DNA in respiratory specimens from patients with chronic obstructive pulmonary disease by quantitative PCR**

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**Purpose:** Data from a previous study suggested a possible association between active EBV infection and Chronic Obstructive Pulmonary Disease (COPD). A cross-sectional study to investigate this observation was undertaken.

**Methods:** Patients with early clinical symptoms of COPD and controls ('healthy smokers' with normal spirometry) were recruited. Combined nasal/oropharyngeal swabs and induced sputums were obtained from each subject. Total nucleic acids were extracted from these specimens, and a TaqMan qPCR assay was used to detect and quantify EBV DNA. Specimen extracts were also tested for the presence of 8 human herpes viruses (HSV-1, HSV-2, VZV, CMV, EBV, HHV-6A, HHV-6B, HHV-7) using the Mobidiag® Prove-it® Herpes TubeArray platform.

**Results:** EBV DNA was detected significantly more often amongst the COPD group (23/45 swabs and 33/43 sputums) than the control group (13/45 swabs and 20/42 sputums).  $P=0.052$  and  $0.007$  for swabs and sputums respectively (Fisher's Exact). EBV copy numbers varied widely (over a 4-log range for swabs and a 5-log range for sputums) and there was no significant difference in geometric mean copy number between the study groups. Data from the Prove-it® TubeArray system will also be presented.

**Conclusion:** Active EBV infection was more common in the disease group than the control group, although there was no association with virus copy number.

**P.060****Influenza A viruses host ultrastructural nuclear modifications: specific different patterns between avian and human strains?**

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**Purpose:** During the 1970s, electron microscopy investigations revealed that human influenza induced drastic modifications of the architecture and the molecular composition of the host cell nucleus and cytoplasm during infection (reviewed in Josset et al., 2008). Many other viruses induce such important remodeling in order to lead optimal infectious cycle (Hiscox et al., 2007). Our purpose was to characterize and compare the host ultrastructural modifications induced by human and avian influenza A viruses in order to correlate these phenotypes with their virulence and host adaptability.

**Methods:** We infected human pulmonary epithelial A549 cell line and Chick Embryo Fibroblast primary cells (CEF) with human (A/NewCaledonia/20/99 H1N1 and A/Moscow/10/99 H3N2) and avian (A/Finch/England/2051/94 H5N2, A/Turkey/582/2006 H5N1 and A/chicken/Belgium/2003 H7N7) viruses. Electron microscopy investigations focused on nuclear and nucleolar ultrastructural modifications were performed on Epon sections of glutaraldehyde fixed cells.

**Results:** Both avian and human influenza viruses specifically alter the nucleus and particularly the nucleolus of infected cells. The regular functional sub-domains of nucleolus become progressively undetectable during the infection. Moreover, several types of viral induced structures with specific morphology can be observed. Altogether, our observations show two seemingly emerging patterns of remodelled nucleus, depending on the human or the avian virus origin.

**Conclusions:** These specific ultrastructural modifications could probably result from differential hijacking of the host nuclear machinery by influenza viruses. The molecular composition of viral induced structures need to be characterized in order to determine their functional significance in regard to the virulence and the host adaptability of influenza strains.

**P.061****Clinical evaluation of pediatric viral acute respiratory tract infections detected by multiplex real-time PCR**

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**Purpose:** To describe the epidemiology and clinical spectrum of respiratory viruses detected in children with acute respiratory tract infection (ARTI).

**Methods:** Respiratory virus multiplex real-time PCR results were obtained from single specimens of children with ARTI (sampled within 48 hours), presenting to the hospital during the 2005–2006 and 2006–2007 winter seasons. Among children aged 0–36 months, specific viral findings were correlated to presenting symptoms and clinical outcome by multivariate logistic regression analysis.

**Results:** A total of 274 children with ARTI (86.1% 0–36 months; 13.9% 3–17 years) were included, and PCR detected respiratory viruses in 81.8% (224/274) of cases. Among single viral infections, respiratory syncytial virus (25.2%) and rhinovirus (19.3%) were most common and predominantly found in young children (0–36 months) compared to older children (3–17 years) (OR 4.2, 95%CI 1.8–9.9). Other single virus infections included influenza virus (5.8%), parainfluenzavirus (2.6%), human metapneumovirus (2.6%), adenovirus (2.2%) and coronavirus (1.5%). Presenting symptoms did not differentiate between viruses, although fever and cough were more associated to influenza virus and respiratory syncytial virus respectively. Rhinovirus was associated with more apparent life threatening events, apnea and intubation compared to other viruses in children aged 0–36 months (OR 3.2; 95%CI 1.0–10). Additional retrospective PCR on specimens obtained during 2006–2007, yielded human bocavirus (6.9%) and coronavirus HKU1 (0.8%), but no *Mycoplasma pneumoniae* (0%) and *Chlamydia pneumoniae* (0%).

**Conclusions:** Real-time PCR frequently detected respiratory viruses in children with ARTI. Clinical features were similar between respiratory viruses. Rhinovirus was frequently detected and was associated with adverse outcome in young children.

**P.062****Human coronavirus NL63 and 229E seroconversion in children**

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**Goal:** Respiratory tract infections by the coronaviruses HCoV-NL63 and HCoV-229E can lead to hospitalization of young children, immunocompromised persons and elderly. In this study we investigated at which age children are confronted for the first time with an HCoV-NL63 infection and, thus, at which age they seroconvert to HCoV-NL63.

**Method:** We designed a recombinant HCoV-229E and a recombinant HCoV-NL63 nucleocapsid protein ELISA and performed a seroepidemiology survey on longitudinal and cross-sectional sera. The longitudinal sera were collected from 13 newborns, from which multiple time points were available spanning a period of at least 18 months. For the cross-sectional survey we tested sera of 139 children between the ages of 0 to 16 years.

**Results:** In the longitudinal serum samples we observed that all children have maternal anti-NL63 and anti-229E antibodies at birth that disappear within 3 months. Seven of the thirteen children become HCoV-NL63 seropositive during follow up, whereas only 2 become HCoV-229E seropositive. Analyzing the cross-sectional serum samples revealed that 75% and 65% of the children in the age group 2.5–3.5 years are HCoV-NL63 and HCoV-229E seropositive, respectively.

**Conclusion:** HCoV-NL63 and HCoV-229E seroconversion occurs on average before children reach the age of 3.5 years.