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Coxsackie A6 (20%), EV71 (4.8%) and echo 3 (3.4%) were the most commonly detected. Analysis of clinical data showed a significant association between hand-foot-mouth disease (HFMD) and genotypes EV71 (risk ratio, rr13.6; Confidence interval, CI 5.4–33.9) and Coxsackie A6 (rr29.4; CI 17.7–48.8). In 2014, Coxsackie A16 was found in stool samples from one AFP case and EV-D68 was found in respiratory specimens from two AFP cases.

Conclusion: This study shows the seasonal fluctuation in circulating EV genotypes from one year to the next, verified in prevalence switches mainly between Coxsackievirus and echovirus genotypes. In addition to the well-known association between HFMD and EV71, we detected an association between Coxsackie A6 and HFMD, which has of late also been observed in other countries. These findings highlight the importance of continued EV surveillance in order to establish the virulence of circulating and upcoming EV genotypes and appropriately guide specific Public Health recommendations.

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Different epidemiological characteristics of respiratory virus infections in children and adults



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Acute respiratory tract infections (RTI) are mostly caused by viruses and are a leading cause of morbidity and mortality especially in young children, the elderly, and in immunocompromised patients. We analyzed epidemiological characteristics of RTI in a university hospital setting in the winter seasons 2015 and 2016.

Diagnostics of respiratory viruses was performed prospectively with the multiplex PCR “FTD Respiratory Pathogens 21” (Fast-track Diagnostics). This kit allows detection of influenza A and B virus (Flu A and B), respiratory syncytial virus (RSV), metapneumovirus (MPV), adenovirus (AdV), coronaviruses (CoV) 229E/OC43/NL63/HKU1, parainfluenzaviruses (PIV) 1–4, rhinovirus, enterovirus, parechovirus and bocavirus (BoV). Results of respiratory virus testing were analyzed of all samples received from the hospital of the University of Würzburg, Germany, during the period January 2015 to April 2016. For data analysis, the study period was subdivided into first season (January 2015 to June 2015) and second season (July 2015 to April 2016).

During the study period, 4136 respiratory samples from 2905 patients at the university hospital Würzburg were tested for the presence of respiratory viruses by multiplex PCR, 2948 in the first season and 1188 in the second season. The median age of the patients was 50.3 years (range 0.01–98.3). Of the 2905 patients 1139 (39.2%) were children. The male versus female ratio was 1.34:1. The overall positivity rate was 37.8% in the first season, 47.1% in the second season, and 40.4% during the whole study period. The positivity rate in children was significantly higher than in adults (61.4% versus 29.5%). Similarly, the rate of double virus detections was significantly higher in children than in adults (17.8% versus 6.5%). Detection of three or more viruses in one sample was only observed in children (4.6%). In both seasons the leading virus detected in respiratory samples was rhinovirus with 27.9% and 25.4%, respectively, of all positive samples. Rhinovirus was followed by Flu A (21.7%), RSV (15.8%), and PIV (12.9%) during the first season and by RSV (18.8%), CoV (17.4%), Flu A (14.7%), and MPV (12.7%) during the second season.

The virus distributions in both seasons were considerably different between children and adults.

In summary, comparison of results of respiratory virus diagnostics in children and adult populations shows substantial differences, which demonstrates the need and usefulness of multiplex PCR for broad spectrum detection of respiratory viruses.

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Presence of human bocavirus 1 and other viral co-infections in hospitalized children with lower respiratory tract infection in Latvia



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Background: Acute respiratory tract infection (ARTI), especially lower respiratory tract infection (LRTI), is the common cause of illness and hospitalization in children worldwide. However, in many cases the etiological agent of disease is unknown. The viruses primarily associated with respiratory tract infections in children are respiratory syncytial viruses, influenza viruses, parainfluenza viruses, adenoviruses, coronaviruses, rhinoviruses and enteroviruses. In recent years the role of several new respiratory viruses in respiratory tract diseases have been reported, including human metapneumovirus, four coronaviruses (SARS-CoV, HCoV-NL63, HCoV-HKU1, MERS-CoV) and human bocavirus 1 (HBoV1). The aim of this study was to determine the presence of HBoV1 and 18 other respiratory viruses in nasopharyngeal aspirates (NPAs) from hospitalized children with LRTI in Latvia.

Material and methods: Forty four children (28 male and 16 female) aged one to 50 months who were hospitalized in Children's Clinical University Hospital and fulfilled WHO LRTI criteria plus had fever ($T \geq 38.0^\circ\text{C}$) were enrolled in this study. In all cases the etiological agent of the disease was not revealed using standard routine clinical methods. NPAs from all patients were obtained on admission and DNA from NPAs was extracted using phenol-chloroform method. All 44 DNA samples were tested for HBoV1 and 18 other respiratory viruses (influenza viruses A, A-H1, A-H1pdm09, A-H3 and B, respiratory syncytial viruses A and B, adenovirus, enterovirus, parainfluenza viruses 1–4, metapneumovirus, rhinovirus, coronaviruses NL63, 229E and OC43) using multiplex real-time PCR method.

Results: Among 44 patients with LRTI, 29 (65.9%) were positive for HBoV1 which was the most frequently detected virus in patients. However, only HBoV1 genomic sequence without any analysed coinfection was detected in two out of 29 (6.9%) patients. Respiratory syncytial virus A was found in 23 out of 44 (52.3%) DNA samples and it was the most common co-infection. Other respiratory viruses detected were: adenovirus in 14 (31.8%), rhinovirus in 9 (20.5%), respiratory syncytial virus B in 7 (15.9%), metapneumovirus in 3 (6.8%), parainfluenza virus 3 in 2 (4.5%), coronavirus 229E in 2 (4.5%), enterovirus in 1, influenza A virus in 1, influenza B virus in 1, coronavirus OC43 in 1 and coronavirus NL63 in 1 patient with LRTI. In 13 cases presence of more than two respiratory pathogens were found and in two cases, none of the tested respiratory viruses were detected.

Conclusion: HCoV DNA is frequently found in NPAs from children with LRTI in Latvia. Although very often HCoV infection is accompanied by co-infections with other respiratory viruses, however there are LRTI cases when HCoV is the only pathogen detected, indicating its possible role in etiology of the disease.

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Molecular epidemiology of circulating human coronaviruses in children at a tertiary hospital in Catalonia (Spain) from 2014 to 2016



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Background: Human Coronaviruses (HCoVs) are single-stranded, positive-sense RNA viruses. Four HCoVs species (229E, OC43, NL63 and HKU1) are currently associated with asymptomatic or mild upper-respiratory tract infections (URTI) in general population, but severe acute respiratory infection (SARI) may occur in patients with high risk of infection, such as immunocompromised patients. The main aim of this study was to describe the seasonality and genetic diversity of HCoVs, and the clinical features related to HCoVs infection, in paediatric patients attended in our hospital from 2014 to 2016.

Methods: From October 2014 (week 40) to May 2016 (week 20) respiratory specimens were collected from paediatric patients who were attended at the emergency care unit, outpatient departments or admitted to Hospital Universitari Vall d'Hebron (Barcelona, Spain) for diagnosis of respiratory viruses by Anyplex II RV16 Detection Kit (Seegene, Korea), that is only able to detect HCoV-229E, HCoV-OC43 and HCoV-NL63, in addition to other respiratory viruses. Partial RNA-dependent RNA polymerase gene (RdRp) was sequenced from laboratory – confirmed HCoVs specimens for subsequent phylogenetic analysis in order to confirm the routine diagnostic PCR results. In addition, partial coding sequence of the spike (S) glycoprotein was sequenced to identify the different HCoV genotypes. Clinical and epidemiological features of HCoV infected cases were retrospectively reviewed from medical records.

Results: A total of 6661 specimens from 3900 patients were received at our laboratory, of which 117 (2%) from 96 patients were positive for HCoVs (11 for HCoV-229E, 12%; 33 for HCoV-NL63, 34% and 52 for HCoV-OC43, 54%). But, phylogenetic analysis of 61 partial RdRp sequences revealed that viruses were belonging to the four species (6 HCoV-229E, 9%; 15 HCoV-NL63, 25%; 22 HCoV-OC43, 36%; and 18 HCoV-HKU1, 30%). HCoVs circulated throughout the year, but highest number of detections were shown in autumn months. Based on phylogenetic analysis of 69 S sequences: HCoV-NL63 (32) fell into two clusters (16 A, 50%; 16 B, 50%); HCoV-OC43 sequences (19) in two clusters (5 B, 26%; 14 C, 74%); and HCoV-HKU1 (18) mainly in other two (16 A, 90%; 1 B, 5%), but one (5%) out of known genetic subgroups.

HCoV was more often found in respiratory samples of children with URTI: 58% had URTI, of which 21% were associated with lower respiratory tract infection (LRTI); 20.5% of patients had LRTI without URTI; and, 21.5% were asymptomatic. HCoV-HKU1 (20%) and

HCoV-OC43 (29%) URTIs were less associated with LRTI than HCoV-229E (50%) and HCoV-NL63 (40%). Most of children admitted with HCoV LRTI required supplemental oxygen (11 out of 17 hospitalised patients), but only 2 required it for more than 4 days. HCoV-229E was related with more oxygen requirements, and HCoV-OC43 with longer hospitalization stays. Only one case was admitted to Paediatric Intensive Care Unit. No fatal cases due to HCoV infection were reported.

Conclusions: Simultaneous circulation of the several HCoVs species was shown from 2014 to 2016. Phylogenetic analysis revealed the circulation of viruses belonging to different genetic subgroups. Despite seasonal infection by these four HCoV species is usually related to mild-respiratory disease, little differences in the clinical features per specie were shown. Virological surveillance must be done to detect changes on the virological and clinical features related to circulating viruses.

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No substantial circulation of enterovirus D68 in patients with severe respiratory disease in South-eastern Spain (Valencian Community) during the 2015–2016 influenza season



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Background: Enterovirus-D68 (EV-D68) was associated with severe respiratory disease in North America and other geographical regions during the fall of 2014.

Methods: We compared the detection rates of EV-D68 in the 2014–2015 influenza season with that of the 2015–2016 season in samples collected in a prospective surveillance scheme for all hospitalizations due to respiratory disease in our region (Valencian Community, South-eastern Spain). Combined nasopharyngeal and nasal (children <14 yr. old) or nasopharyngeal and pharyngeal swabs are analyzed in a single laboratory at FISABIO-Public Health for 16 respiratory viruses by multiplex real-time RT-PCR, including rhinovirus/enterovirus as a single target. All samples positive for rhinovirus/enterovirus were retested with a rhinovirus/enterovirus discriminative real-time RT-PCR, and those enterovirus positive for EV-D68 specific detection as a single target.

Results: In the 2014–2015 season, between November 15th and March 31st, 372 of 4472 (8.32%) samples were rhino/enterovirus positive, of which 66 (17.75%) were identified as enterovirus, and 15 (4.03%) confirmed as EV-D68. In the 2015–2016 season, between November 15th and April 30th, 201 of 2700 (7.45%) samples were rhino/enterovirus positive, of which 42 (20.82%) were identified as enterovirus, and only one (0.50%) confirmed as EV-D68.