**Conclusion:** HBoV1 DNA is frequently found in NPAs from children with LRTI in Latvia. Although very often HBoV1 infection is accompanied by co-infections with other respiratory viruses, however there are LRTI cases when HBoV1 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 singlestranded, 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.