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0.7.5

Human bocavirus infections have seasonal transmission dynamics

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Purpose: Human bocavirus is a common finding in children with respiratory infections. It is often found together with other viruses and its role as the primary cause of the symptoms is not completely clear. The aim of this study was to determine the incidence, clinical features, and impact of bocavirus infections in a large cohort of Finnish children.

Methods: The cohort consisted of 1338 children <13 years of age, who were prospectively followed for any signs of respiratory infection during two periods between October and May in 2000–2002. At each new visit to the study clinic, a nasal swab was collected for virological diagnosis. Bocavirus DNA was detected in stored nucleic acid extracts using real-time PCR with a dual-label probe. We tested a total of 2930 specimens including every specimen from the first three months of the study and every third specimen thereafter.

Results: There was a clear periodicity of the appearance of bocavirus infections in the study population. The highest prevalence of 23.1% was observed in November 2000 and the lowest prevalence of 0.8% in October 2001. High prevalence (>10%) was observed in October 2000-January 2001 and December 2001 – March 2002. The monthly incidence of specimens with a high copy number (>10000 copies/swab) was 0.0–6.4%, and was not a constant proportion of the overall prevalence.

Conclusion: There is a clear seasonal forcing factor influencing the appearance of bocavirus infections in child population. At times of peak incidences, bocavirus may be a major cause of respiratory infections in children.

0.7.6

Enhanced etiological diagnosis of respiratory virus infections and outbreaks using nucleic acid amplification testing against an expanded range of targets

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Purpose: This study was undertaken to evaluate the performance of the Luminex xTagTM Respiratory Viral Panel (RVP) assay (Luminex Molecular Diagnostics) for respiratory virus diagnosis and outbreak investigations.

Methods: Direct fluorescent antigen (DFA) testing was used as an initial screen for influenza virus (IFV)A, IFVB, parainfluenza (PIV)1–3 and respiratory syncytial virus (RSV) on nasopharyngeal (NP) samples. DFA-negative NP samples and all other sample types were subjected to nucleic acid extraction using the easyMAG® extractor and reagents (bioMérieux Ltd). In the initial evaluations, both in house nucleic acid amplification tests (NATs) and RVP was undertaken (n = 1497). In a separate analysis over 2006/2007, 1108 samples from 244 outbreaks were analyzed by DFA, in house NATs and RVP. From February 2008 RVP was implemented as the front-line NAT with more than 5,000 samples analyzed to date.

Results: Results for targets included in our in house NATs and RVP were highly concordant (Kappa 0.73–1.00). Addition of RVP to the testing algorithm increased the etiological diagnosis for outbreaks to >80% for 2006–2008, mainly because of enhanced identification of rhinoviruses and coronaviruses outside of the classic winter season. RVP proved to be more cost effective than undertaking individual or small multiplex real-time NATs to cover the full range of respiratory virus targets.

Conclusion: The Luminex RVP assay allows for efficient, cost-effective, multiplex detection of a broad range of respiratory viruses which enhances our etiological diagnosis of respiratory virus infections and outbreaks.

0.7.7

Impact of duration of illness on viral load and detection rate of viral pathogens by multiplex PCR in respiratory tract infections

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Purpose: We have evaluated a multiplex PCR method for rapid detection of viral and bacterial pathogens in adult patients (>18yrs) with symptoms of RTI for less than two weeks

Methods: Throat and nasopharyngeal swabs from 219 out-patients along with 100 asymptomatic controls were obtained during two winter seasons (Oct-Apr 2006–07 and 2007–08). The following agents were analysed; Rhinovirus, Coronavirus (229E, OC43, NL63), Influenza A & B virus, Parainfluensavirus 1–3, Human metapneumovirus, RS-virus, Adenovirus, Enterovirus, *M. pneumoniae* & *C. pneumoniae*. Clinical and demographic data were recorded according to a standardised questionnaire.

Results: We found a positive PCR result in 43% of the patients. In 7% of the patients more than one agent was detected. The diagnostic yield was significantly higher if samples were taken within the first 6 days of illness rather than after 6 days (51% positive vs. 30% positive, p<0.01). For Corona virus and Influenza A & B virus we found an association between the amount of virus detected, measured as CT-value, and duration of illness (n=13, rs=0.33, p<0.05; n=24, rs=0.17, p<0.05; and n=10, rs=0.65, p<0.01, respectively). Only 2% of asymptomatic controls were positive by PCR for any pathogen, indicating that a positive result by the multiplex PCR assay was clinically relevant.

Conclusion: We conclude that the duration of symptoms should guide the clinician in how to use molecular tests for virus detection in the RTI setting. The quantity of virus found may determine the clinical relevance of a positive PCR result, at least for some of the investigated pathogens.

O.7.8

Burden of disease due to human coronavirus NL63 infections in children under 3 years of age

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Goal: The disease burden caused by the recently identified respiratory viruses – like HCoV-NL63 – is unknown. We determined the burden of disease due to HCoV-NL63-infections using samples of the PRI.DE study. In this population-based PRI.DE cohort, children under the age of 3 with lower respiratory tract infections (LRTIs) are included and one can calculate the annual incidence rate and the total national disease burden attributable to HCoV-NL63-related LRTIs.

Method: In total 1756 respiratory samples, from hospitalized children with LRTIs, or children with LRTIs who visited the outpatient clinic, were tested. HCoVNL63 positive samples were detected using a real-time RT-PCR tool and only single HCoV-NL63-infections with high virus load (>10.000 copies/ml) were included in further analysis.

Results: For outpatients, the annual incidence of HCoV-NL63 infection was calculated to be 7 per 1000 children per year (95% confidence interval (CI) 3–13 per 1000 children per year), reflecting an absolute number of 16,929 visits to the physician in Germany per year. The estimated hospitalization rate due to HCoV-NL63 alone is 22 per 100,000 children (95% CI: 7–49 per 100.000 children per year). This number reflects only 522 children in Germany per year.

Conclusion: This is the first study that visualizes the impact of HCoV-NL63 infection in young children with LRTIs and illustrates that an HCoV-NL63 infection in children below 3 years of age often requires a visit to the physician in an outpatient clinic, but hospitalizations are relatively infrequent.