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PIV-25

Phylogenetic analysis of Italian human metapneumovirus (HMPV) and human bocavirus (HBOV) strains

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Purpose: To analyze phylogenetically hMPV and hBoV strains detected during a surveillance study among 240 children hospitalized with Acute Respiratory Infections in Milan (Northern-Italy) from 2004 to 2008. During this study 20 (8.3%) and 29 (12.1%) samples (oropharyngeal swabs) resulted positive for hMPV-RNA and hBoV-DNA, respectilvely.
Methods: A representative number of positive-samples (hMPV=10, hBoV=16) was then selected for sequencing. HMPV/F-gene and hBoV/VP2-gene sequences were aligned (ClustalX) with others (hMPV=25, hBoV=28) obtained from GenBank. Pyhlogenetic trees (Neighbor-Joining method, Kimura 2-Parameter model, 1000 bootstrap replicates) were constructed and evolutionary mean distances between/within groups were calculated (MEGA4.0). Recombination events and breakpoints were detected through Bootscanning analysis (Simplot3.5.1).
Results: 8 hMPV Italian sequences belonged to genotype A, whereas 2 belonged to genotype B. According to evolutionary mean distances between/within groups analysis, 4 different lineages (A1/A2-B1/B2) and two further sub-lineages in A2-cluster (A2a/A2b) were identified (figure).

A

Within Analysis					
Taxa	Mean Distance	Standard Error	Mean Similarity	Similarity Range	
A2b	0.0100	0.0044	0.99	0.974	1
A2a	0.0113	0.0053	0.989	0.984	0.994
A1	0.0325	0.0073	0.967	0.954	0,984
B2	0.0124	0.0049	0.988	0.984	0.989
B1	0.0123	0.0047	0.988	0.979	0.994

B

Between Analysis								
	Mean distances				Similarities			
Taxa	A2b	A2a	A1	B2	Taxa	Similarity	Similarity Range	
A2a	0.0281				A1 vs A2a	0.921	0.913	0.938
A1	0.0855	0.0787			A1 vs A2b	0.915	0.897	0.933
B2	0.1814	0.1732	0.1804		A2a vs A2b	0.972	0.954	0.984
B1	0.2087	0.1977	0.1692	0.0825	B1 vs B2	0.9175	0.913	0.938

Evolutionary mean distances over all sequence pairs within (A) and between (B) distances were calculated by Kimura 2-parameter model with MEGA4 software.

Italian sequences were then classified as: A2b (N = 6), A2a (N = 1), A1 (N = 1), B1 (N = 2).
10 hBoV Italian sequences belonged to genotype St2, 4 belonged to genotype St1 and 2 (100% identical) were located outside the 2 main branches. Bootscanning analysis of those sequences detected a recombination event: breakpoint was located on nt1302 of VP1/VP2 gene.
Conclusions: Two possible hMPV-A classifications have been proposed (Huck, 2006; Escobar, 2009): our results indicate the existence of two different clusters within A2 lineage, in agreement with Huck.
Recombination among hBoV strains suggests that recombination plays an important role in the evolutionary history of these viruses.

PIV-26

Human bocavirus-monoinfection and viremia are associated with airway infection in children

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The discovery of human bocavirus (HBoV), a parvovirus, was published in 2005. It has been associated with airway infections in children, and relations to more complex clinical conditions in immunosuppressed patients have been suggested. We here present a controlled clinical study of HBoV in children with airway infection.
Material and Methods: Nasopharyngeal aspirates were collected from children admitted to hospital with respiratory tract infections during the time period June 6, 2007 to February 28, 2009. The control group consisted of children admitted to elective surgery.
Using PCR the nasopharyngeal aspirations were examined for HBoV and 16 other viruses and bacteria.
Results: Total material: 1015 samples (873 patient samples and 142 controls).
10% of the patient samples and 16% of the control samples were positive for HBoV. The difference was not significant when we corrected for the age difference in the two groups.
The prescense of HBoV without other viruses in the same nasopharyngeal aspirate was significantly associated with airway symptoms. Viremia was found almost exlusively in patients under the age of two. No children in the control group had viremia. Low age, high viral load and presence of viremia were each associated with a greater occurence of LRTI.
Multiple infections were found in 76% of the samples positive for HBoV.
Conclusions: Human bocavirus is common among healthy children. Our data support previous studies indicating that HBoV is a cause of airway infections in children. Viremia and the prescence of HBoV-monoinfection may be helpful markers for clinical relevance when HBoV is detected.

PIV-27

Viral pathogens of respiratory tract infections in Ankara, Turkey

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Respiratory tract infections are caused by various viruses. They are one of the major causative agents of upper and lower respiratory tract illness with annual winter outbreaks. In this study we aimed to documented the viral etiology of acute respiratory tract infections in Ankara.
Samples from patients with respiratory tract infections have been analysed for viral pathogens during January-May 2009. Nasal swabs, nasopharyngeal swabs and aspirates were collected from hospitalized patients. Influenza A, Influenza B, Parainfluenza, Rhinovirus, Coronavirus, Respiratory syncytial virus, Human metapneumovirus and Adenovirus were detected with both commercial multiplex PCR (Fast-track Diagnostics, Luxemburg) and cell culture based indirect immunofluorescence assay.
74 clinical specimen were sent to laboratory from different hospitals during January-May 2009. 48 sample were found positive at least one of the respiratory viruses. The predominant virus was RSV (45.2% of total positive detections). RSV, Adenovirus, Rhinovirus and Parainfluenza viruses predominated in younger children while Influenza A and Influenza B were more prevalent in adults. The peak frequency of respiratory tract viruses had a peak in early spring. Adenovirus, Rhinovirus and Coronavirus coinfections were determined with RSV infections, and one Coronavirus coinfection was ascertained with Influenza A infection. Wide range of viruses can cause respiratory tract infections. RSV were found the most common pathogen causing respiratory tract infection in hospitalized children and Adenovirus were second only to RSV.

PIV-28

Detection of respiratory viruses by molecular methods – a way of improving conventional diagnosis

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The clinical presentation of different viral respiratory infections can be very similar, therefore rapid and accurate etiological diagnosis is necessary. This study compared two multiplex RT-PCR assays for simultaneous detection of different respiratory viruses to conventional methods, cell culture (CC) and immunofluorescence assay (IF). Viruses detected by both PCR assays (Seeplex® RV12 ACE Detection kit, Seegene and CLART® Pneumovir kit, GENOMICA S.A.U.) were: influenza A and B viruses, parainfluenzavirus 1, 2 and 3, adenoviruses, respiratory sincitial viruses A and B, human metapneumovirus, human coronaviruses and rinoviruses. Additionally the CLART® kit detected influenza C virus, parainfluenzavirus 4, bocavirus and enteroviruses. Eighty nasopharyngeal aspirates obtained from children