P2172

Human Enterovirus and Human Rhinovirus Acute Respiratory Tract Infections in Hospitalized Pediatric Patients, France, 2009-2010

F. RENOIS[1]*, N. LEVEQUE[1], P.G. DELIEGE[1], A. BOUIN[1], C. FICHEL[1], M. ABELY[2], Y. NGUYEN[1], L. ANDREOLETTI[1]

[1]Clinical and Molecular Virology Unit, CHU and EA-4684 Medicine Faculty of Reims, France;

[2]Pediatric Unit A, American Memorial hospital, University Hospital Centre, Reims; France.

* frenois@chu-reims.fr

Introduction and purpose

The *Enterovirus* (EV) genus (*Picornaviridae* family*)* includes 7 are pathogenic for humans: 4 species (A-D) of human enterovirus (HEV) and 3 species (A-C) of human rhinovirus (HRV) (Lauber et al., 2012). Among the EV genus, HEV and HRV are recognized as leading causes of Acute Respiratory Tract Infections (ARTIs) in humans (Jartti et al., 2004, 2012 ; Antunes et al., 2010; Jacques et al., 2006).

Recently, the use of new molecular tools have demonstrated a new specie HRV-C that could be more specifically responsible for lower ARTIs in pediatric patients (Lau et al., 2007; Miller et al., 2009; Tapparel et al., 2011) but also new HEV respiratory genotypes have emerged or re-emerged, (e. g. HEV-68, 104, 109, 117 and CVA-21) (Piralla et al., 2012; Yozwiak et al., 2010; Ikeda et al., 2012; Xiang et al., 2012; Kaida et al., 2011, 2012; Pankovics et al., 2012; Daleno et al., 2012).

The HEV and HRV genotypes detection in the respiratory tract samples remains difficult even using molecular tools because of the large EV genetic diversity, leading to an underestimation of the prevalence and role of HRV and HEV in pediatric ARTIs (Jartti et al., 2012). The detection, identification and monitoring of EV genotypes in ARTIs would be helpful to understand the (re)-emergence of strains responsible for epidemics characterized by severe respiratory symptoms (Jartti et al., 2012).

Purpose: To assess the etiological role and the clinical characteristics of HRV and HEV infections in pediatric patients hospitalized for acute respiratory tract infections (ARTIs).

Patients & Controls

Of the 1195 patients hospitalized in the pediatric department of the University Medical Centre of Reims (Champagne-Ardennes, France) from September 2009 to June 2010 who underwent nasopharyngeal aspirations (NPAs), 519 were retrospectively selected because they were negative by use of classical virological and bacterial tests (Jacques et al., 2006; Bouscambert et al., 2005) (Fig 1). Of these 519 hospitalized patients, 309 were admitted for ARTIs and 210 without any clinical signs consistent with ARTIs were considered as controls patients hospitalized for various non-respiratory diseases and systematically sampled for prevention and control of nosocomial viral infections in paediatric wards (Fig 1).

Informed consent was obtained from the infants' parents and the hospital's ethics committee (Institutional Review Board of the Reims University hospital) approved the present study.

Methods

Total nucleic acid extraction (DNA and RNA) was retrospectively performed from each respiratory specimen using the NucliSens EasyMAG instrument (BioMerieux, Lyon, France) according to the manufacturer's instructions.

RT-qPCR assays (specific « Pan-entero-rhino » (Tapparel et al., 2009) and specific HEV assays (Petitjean et al., 2006) and molecular sequencing methods (VP4-VP2 capsid gene (Savolainen-Kopra et al., 2009)) were used to identify HRV and HEV strains.

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The work presented shows the results of two publications of our team:

Renois F, Lévêque N, Deliège P-G, Fichel C, Bouin A, Abely M, Nguyen Y, Andréoletti L. Enteroviruses as Major Cause of Microbiologically Unexplained Acute Respiratory Tract Infections in Hospitalized Pediatric Patients. J of Infection. 2013.

Renois F, Bouin A, Andréoletti L. Enterovirus 68 in Pediatric Patients Hospitalized for Acute Airway Diseases, 2009-2010, Reims, France. J Clin Microbiol. 2013. 51:640-3.

Results

Figure 1 : Study design and frequency of detection of human enterovirus and human rhinovirus strains by Real-Time RT-qPCR in Nasopharyngeal aspirations (NPAs) samples of pediatric patients hospitalized for acute respiratory tract infections from September 2009 to June 2010 in Northern east of France.

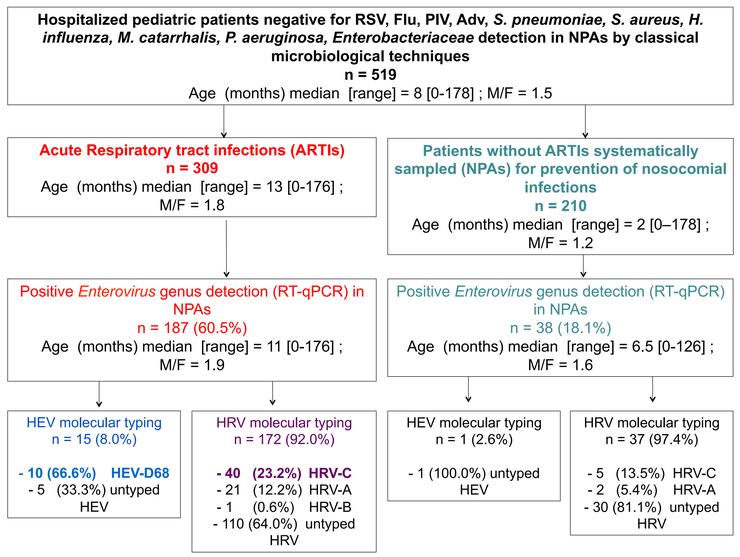


Figure 2: Molecular phylogeny of Enterovirus based on the partial VP4/VP2 region nucleotide sequence.

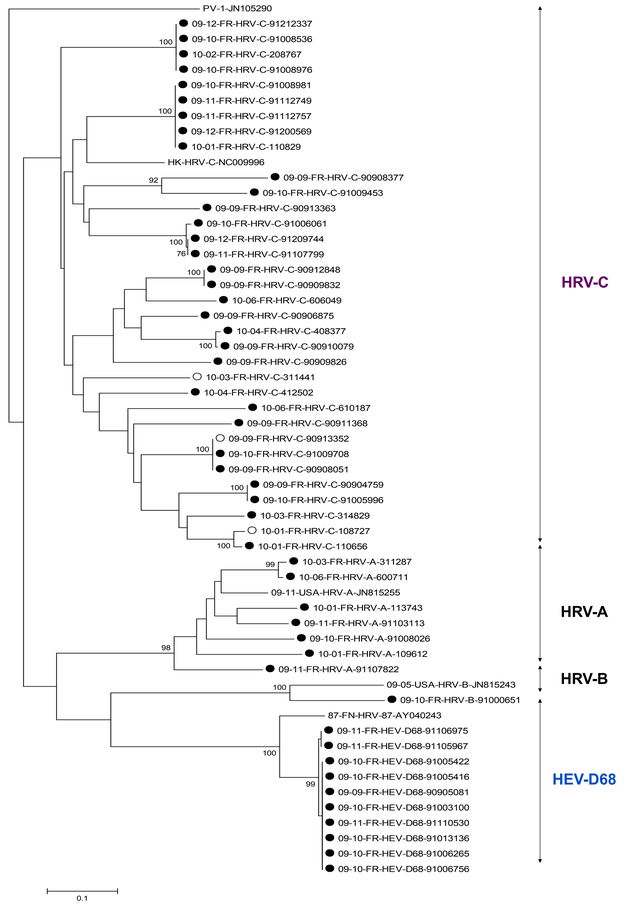


Table 1 : Clinical characteristics of pediatric patients hospitalized for microbiologically unexplained bronchiolitis or exacerbated asthma and positive for human enterovirus or human rhinovirus detection of by Real-time RT-qPCR in nasopharyngeal aspiration (NPAs) samples.

	HEV	HRV	md	*P*
Total number of strains n= (%)	12 (100.0%)	121 (100.0%)	0	
Age (months) median [range]	36 [6-83]	11 [0-155]	0	**0.003**
Sex ratio (M/F)	2	2.3	0	0.99
Length of hospitalization (days) median [range]	3 [1-5]	3 [0-22]	2	0.6
Admission in Intensive Care Unit n= (%)	0	4 (3.3%)	0	0.99
Clinical outcome : number of death n=	0	0	0	
Medical history :				
Asthma or infantile asthma* n= (%)	6 (50.0%)	44 (36.3%)	0	0.36
Prematurity ± n= (%)	3 (25.0%)	21 (17.3%)	37	0.70
Passive smoking n= (%)	2 (16.6%)	31 (25.6%)	70	0.24
Family atopic history n= (%)	6 (50.0%)	58 (47.9%)	49	0.23
Symptoms at the time of admission :				
Fever§ n= (%)	8 (75.0%)	43 (35.5%)	12	0.12
Respiratory distress¶ n= (%)	11 (91.6%)	62 (51.2%)	2	**0.01**
Need for Oxygen therapy n= (%)	8 (75.0%)	49 (40.4%)	17	**0.01**
Viral load per ml of NPAs samples at the time of admission : Median values copies / ml [range]	$7.4x10^6$ [$2x10^5$ - $5.4x10^7$]	$4.9x10^7$ [$2x10^5$-$7.2x10^{10}$]	0	**0.005**

md: missing data

* Asthma is defined as one episode of wheezing dyspnea in a child of more than 2 years. Infantile asthma is defined as more than two episodes of wheezing dyspnea in a child of less than 2 years.

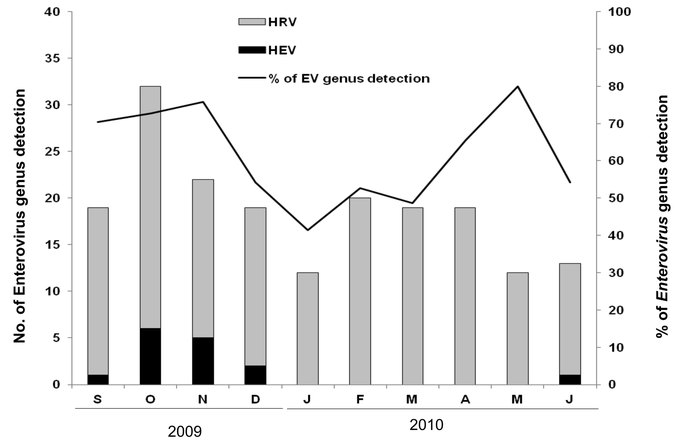
± Prematurity is defined as a birth occuring before 37 weeks of amenorrhea.

§ Fever was defined as the presence of an external body temperature > or = to 38°C.

¶ Respiratory distress was defined as the presence of dyspnea associated with one of the following clinical symptoms: chest indrawing, accessory respiratory muscle use (e.g scalene muscles), paradoxical breathing or nasal flaring.

Statistical significant values (P<0.05) are shown in bold.

Figure 3. Seasonal distribution of human enterovirus (HEV) and human rhinovirus (HRV) detection in Nasopharyngeal aspirates samples (NPAs) of pediatric patients hospitalized for acute respiratory tract infections (ARTIs) from September 2009 to June 2010.



Total number of sample tested (n = 309)	27	44	29	35	29	38	39	29	15	24
Number of Positive EV genus (n = 187)	19	32	22	19	12	20	19	19	12	13

Conclusions

In conclusion, HRV and HEV strains were identified as potential etiological causes of 60.5% of microbiologically unexplained ARTIs diagnosed in hospitalized pediatric cases. A higher clinical severity was observed in HEV-induced bronchiolitis or asthma exacerbation cases in comparison to HRV-related similar cases during the study period. The present work highlights the need to perform a broad HEV and HRV molecular detection in the respiratory samples of pediatric patients hospitalized for lower ARTIs, which results could improve the clinical and therapeutic management of children hospitalized in pediatric wards.