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NON-INFLUENZA VIRUSES IN ACUTE RESPIRATORY INFECTIONS AMONG YOUNG CHILDREN. HIGH PREVALENCE OF HMPV DURING THE H1N1V.2009 PANDEMIC IN POLAND

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ABSTRACT

In Poland the majority of hospitalized cases of pneumonia (annually more than 70000) were reported without determination of an aetiological agent (J18 of ICD-10), also because diagnosis of viral ARTI is limited to identification of influenza viruses or sometimes RSV.

MATERIAL AND METHODS. For determination the contribution of non-influenza viruses in ARTI among children, 381 nasopharyngeal swabs from hospitalized in period X.2008-IV.2011y. children (aged 1 day – 5 y.o.) were tested for RSV, HMPV, HEV/HRV, HPIV 1-3, HAdV, HBoV.

RESULTS. At least one viral agent was detected in 72.7% of patients. The most predominant was RSV infection (49%), followed by HEV/HRV (15.5%); HMPV (8.7%), Adenoviruses (7.4%), HPIVt.1-3 (5.8%) and HBoV (5.5%). Seven periods based on the median of examined children/month were determined: 3 with increased number of ARTI. RSV infections, diagnosed in all periods, were predominate in five periods, mainly in LRTI cases. In the 3thperiod - HMPV was predominant, in the 5th – HEV/HRV. It was found that clinical manifestation of HMPV infections varied depending on the period.

CONCLUSIONS. Relatively high prevalence of HBoV or HMPV cases of ARTI, especially different clinical picture in some periods (ARTI without pneumonia or bronchiolitis), indicated necessary of more detailed molecular and epidemiological studies. Also our results indicate the need for improved diagnostic capabilities of virological tests in acute upper and lower respiratory tract infections in children.

Key words*: acute viral respiratory tract infection in young children; seasonality; activity of RSV and HMPV and HBoV;*

INTRODUCTION

Diseases of respiratory tract, including infections, are one of the main reasons of annual hospitalization in Poland (7-8%). Among babies and children this ratio is usually higher: in children aged 1 – 4 about 30%, among infants - 15-17% (J00-J99 according to International Statistical Classification of Diseases and Related Health Problems, 10th Revision [ICD-10]). In recent years, respiratory infections per year were approximately 40% of all hospitalizations associated with respiratory diseases (in 2009 - 43.6%; in 2010 – 42.7%.). However, this ratio reached 90% in infants (91% in 2009; 89.5% in 2010) or 70% among children aged 1-4 (73.5% in 2009; 72.8% in 2010) (J00-J22 of ICD-10). Different bacteria and viruses may be an agent of acute respiratory tract infections (ARTI). Viruses are very common aetiological agents of pneumonia, bronchiolitis, bronchitis and other respiratory infections. In Poland the majority of hospitalized cases of pneumonia were reported as pneumonia without determination of an aetiological agent (J18 of ICD-10). In 2009 – 75 191 of such cases were reported, in 2010 – 72 694 (1). Diagnosis of viral acute respiratory infections is still a problem. There are some reasons related to difficulties in conducting diagnosis of viral infections: high number of possible aetiological agents, similar symptoms of disease, emerging new variants of described viruses or new viruses and high cost of examinations. Moreover, in Poland diagnosis of viral respiratory infections is very often still limited to

identification of influenza viruses or sometimes RSV. In such a situation, every year in Poland the origin of aetiology for over 50% of cases of acute viral respiratory infection is uncertain. The surveillance of ARTI caused by viruses other than influenza and RS is done in many countries (Canada, USA) and the profile of examined viruses is often upgraded and enlarged (2-4).

The aims of our studies were: 1. to determine the contribution of non-influenza viruses in acute respiratory viral infections among hospitalized infants and children; 2. To analyse the activity of different respiratory viruses tested in particular years, months and seasons of increased number of cases.

MATERIAL

Nasopharyngeal swabs collected from 381 infants and children from the first day of life to the 5th year, hospitalized with acute viral respiratory tract infection between October 2008 - April 2011.

Data on the age of examined children and main clinical symptoms were obtained from 346 patients. Almost all children (96%) were below 3 years old, and the majority of them were below 6 months old (62%) or belonged to 6-12 months age group (30%). The boys amounted to 57.5% of examined children.

METHODS

PCR. Nucleic acids were isolated from all 381 nasopharyngeal swabs using QIAamp Viral RNA mini kit (for prospective examinations) and QIAamp DNA mini kit (257 swabs - selected specimens collected in period I.2009-IV.2011 – retrospective tests). Specific fragments of viral genome were detected by PCR method for DNA viruses: Adenoviruses (HAdV) and Bocavirus (HBoV) or PCR with reverse transcription step (rt-PCT) for Respiratory Syncytial Virus (RSV), Human metapneumovirus (HMPV), Enteroviruses/Rhinoviruses (HEV/HRV) and parainfluenza viruses type 1, 2 and 3 (HPIV t.1,2,3) using primers previously described (5-9).

Detection of viral antigens. HMPV antigen was detected in 273 specimens by Biotrin hMPV Antigen EIA (Ireland) according to manufacturer instruction (10).

Statistical analysis of demographic data, seasonality and detected viruses were done using Statgraphics for Windows, Centurion, v.XV. StatPointTech.Inc.USA. For qualitative/categorical data crosstabulation, tests of independence (χ^2 or Fisher's exact tests), analyses of the degree of association between rows and columns (Contingency Coeff., Lambda test and Pearson's correlation) were conducted. For determination of periods with increased number of respiratory infections the median number of patients examined every month was used.

RESULTS

Generally, at least one viral aetiological agent was detected by PCR and/or antigen test in 277 out of 381 examined patients (72.7%). The highest number of children was infected with RSV – 187/ 381 children (49%), followed by Enteroviruses (Enteroviruses/Rhinoviruses) – 59/381 (15.5%); HMPV – 33/381 (8.7%), Adenoviruses – 19/257 (7.4%), HPIV 1,2,3 – 22/381 (5.8%) and HBoV – 14/257 (5.5%). In specific years,

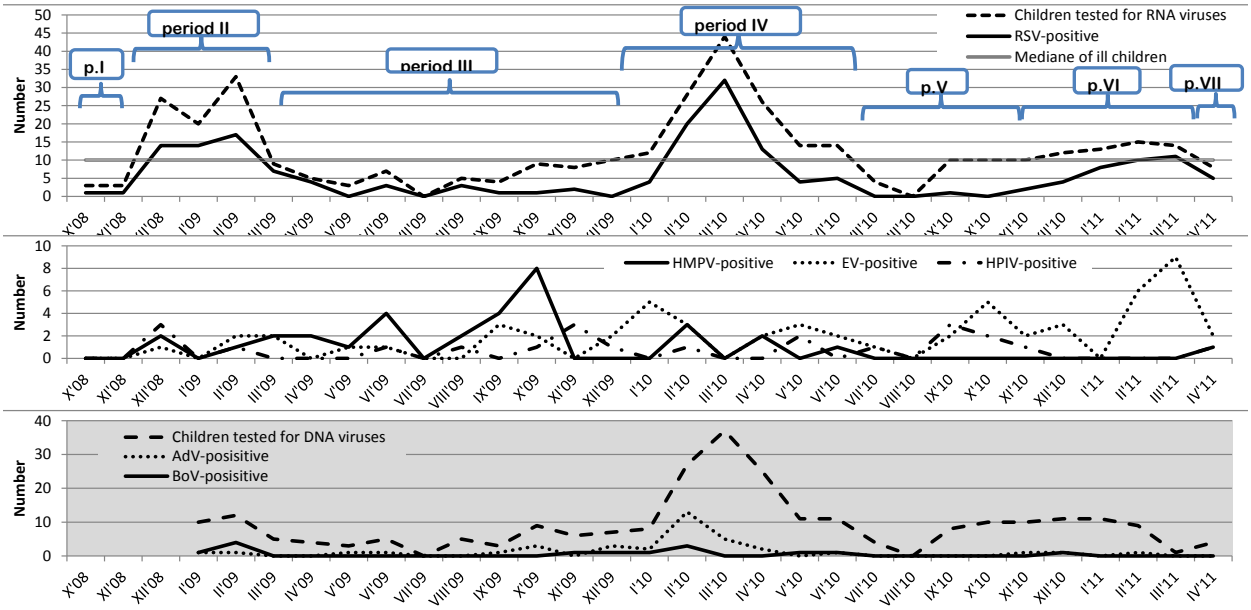


Fig. 1. Monthly distribution of detected viruses (RSV, HMPV, HEV/HRV, HPIV t.1-3, HAdV, HBoV) in children hospitalized in X.2008 and IV.2011. Separated periods based on the number of hospitalized children with suspicion of acute viral respiratory infection.

the distribution of particular viruses was varied and significant differences were mainly found for HMPV (2% in 2011 vs. 21.2% in 2009) and HEV/HRV (2.9% in 2008 vs. 33.3% in 2011). Significant differences in the frequency of adenoviral, bocaviral and HPIV t.1,2,3 infections in different years were not detected (P_o >0.05).

Another study regarding virus distribution by consecutive 31 months indicated that for all viruses, except HBoV, the relation between number of diagnosed cases and months of sample collection was significant (P_o<0.05). The distribution of viruses during the following 31 months of the study is presented on Figure 1. The distribution of identified viruses in consecutive weeks was also analysed. The significant differences (P_o<0.05) in week distribution during all study were found for all viruses, except HAdV. However, there were some differences about the particular years of the study. The highest differences in week distribution of RSV were found in 2010 (P_o=0.0026), for HMPV – in 2009 (P_o=0.0000) and for HEV/HRV – 2011y. (P_o=0.0027).

RSV infections mainly were observed from December to April. In our study, the distribution of RSV during 3 consecutive winter-spring seasons was examined (Fig. 2). The RSV epidemic curve during those 3 seasons varied from sharp curve in the season of 2009-2010 with one-peak in March 2010 and to two-peaks curve in the season of 2008-2009 (peak in December 2008 and February 2009). The epidemic curve of RSV infection observed in the season of 2010-2011 seems to be flat with the low peak in March 2011, however, the possibility of the increased number of RSV infections in May 2011 cannot be rejected.

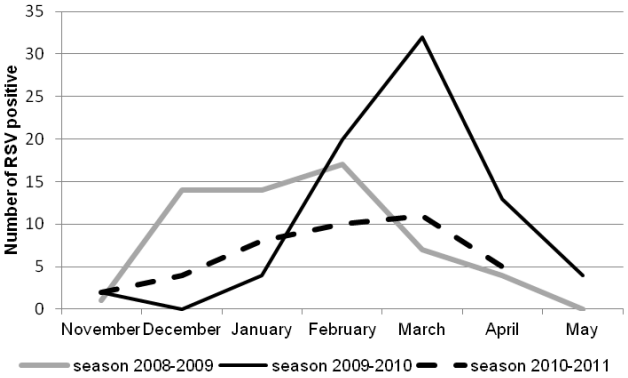
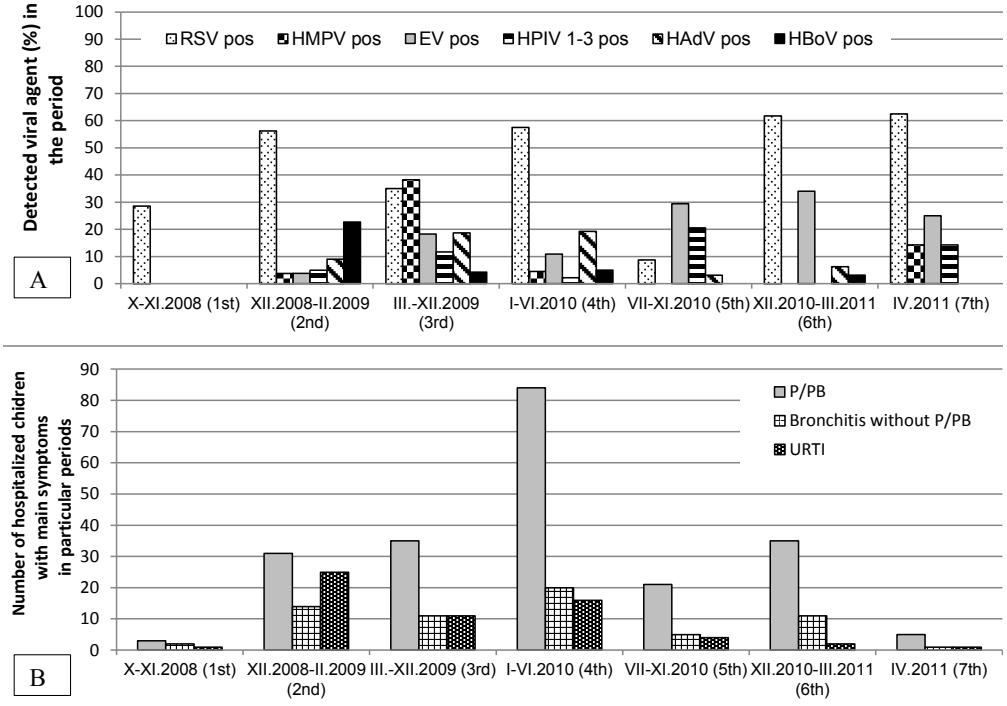


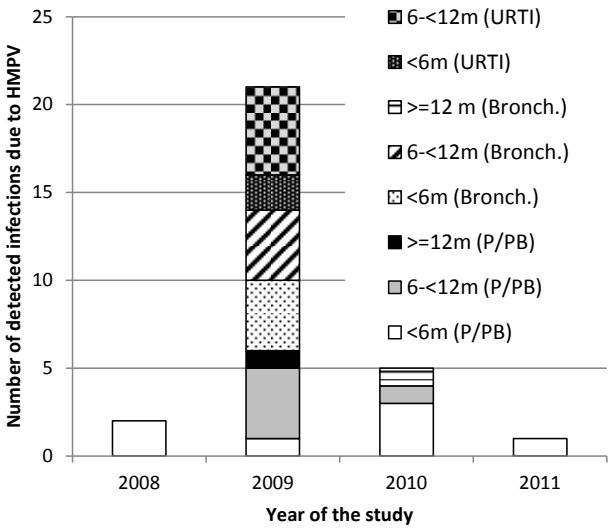
Fig. 2. RSV infections during 3 epidemic seasons 2008-2009, 2009-2010 i 2010-2011

Then, the periods of increased number of acute viral respiratory infections were determined. The division into periods was based on the median of examined babies and children per month (10 children per month) (Fig.1). There were months when the number of examined children exceeded the median of patients per months. Finally, seven periods were determined. There were periods with increased number of hospitalized children: second (XII.2008 – II.2009), fourth (I-VI.2010) and sixth (XII.2010-III.2011). There were periods with lower



Abbreviations: P/PB - pneumonia, bronchiolitis or pneumobronchiolitis cases; Bronchitis witout P/PB – cases of bronchitis only; URTI – upper respiratory tract infection

Fig. 3. Participation (%) of tested viruses (A) and main clinical manifestation (B) in 7 periods separated on the basis of the number of hospitalized children with respiratory tract infections



Abbreviations: URTI – upper respiratory tract infections; Bronch..- bronchitis; P/PB – pneumonia or pneumobronchiolitis

Fig. 4. Infections due to HMPV in period 2008-2011 by age of children and main recognition

number of hospitalized patients: first (X-XI.2008), third (III-XII.2009), fifth (VII-XI.2010) and seventh (IV.2011).

The analysis of percentage of babies and children with identified viral agent of infection, as well as the distribution of particular viruses in particular periods were conducted. The percentage of patients with identified viral agent of infection in the periods was higher than 50% (except the first period- in 2008y.) and reached 87.5% in the 7th period and 85% in the 3rd period. The distribution of tested viruses in particular periods varied. RSV infections were diagnosed in all particular periods. Those infections were predominant in five periods (1st, 2nd, 4th, 6th and 7th). Infections caused by other viruses were predominant in summer and fall time: in the 3th period (HMPV) and the 5th period (HEV/HRV or HPIV). Moreover, high involvement of other viruses was observed also: HAdV – in 3-4th periods and HBoV – in 2nd period. The strongly significant increase in HMPV infections in the 3rd period and HEV/HRV infections in the 6th period was found (Fig.3.A).

The analysis of the main clinical manifestations was also done. It was recognized that pneumonia was the most common (51%) followed by acute bronchitis (19%), acute upper respiratory infections (18%) and bronchiolitis (7%) or pneumobronchiolitis (5%). The recognition of pneumonia was in more than 50% of cases in the following periods: 3th, 4th, 5th, 6th and 7th. In the 1st and 2nd period "other ARTI" (bronchitis and URTI together) were mainly diagnosed, however the ratio of pneumonia in these periods remained also high (Fig. 3.B). The most severe infections of lower respiratory tracts were not numerous. Bronchiolitis and pneumobronchiolitis were significantly more frequently observed in the 4th and 2nd periods. 75% of all bronchiolitis were recognized in the 4th period when very high number of RSV infection was also observed. It should be pointed out that in the periods with increased number of hospitalized children, the higher frequency of more severe LRTI cases was observed.

Acute upper respiratory tract infections were recognized in 60 hospitalized patients. Among them 20 was caused by RSV (only 1.2% of all RSV positive), 7 – by HMPV (23% of all HMPV pos), 7 – by HEV/HRV (14%), 5 – by HBoV (38.5%), 4 - by HPIV t.1-3 (18.2%) and 3 – by HAdV (only 9.4% of all HAdV positive).

Unexpected increase of HMPV infections in 2009 y. required more detailed analysis. In 2009 year, the majority of HMPV infections were found among children from 6 months to 1 y. (60%), and below 6 months (36%). In other periods the majority of children with HMPV infections were below 6 months (75%). In general, the clinical manifestation in children infected with HMPV were mainly upper respiratory tract infections (URTI) – 23%, followed by bronchitis (30%) and pneumonia (27%). However, children with acute URTI caused by HMPV were hospitalized only in 2009 and in the 3th period. This difference was significant (P_o=0.0000) although a small number of cases was analysed (Fig. 4). Also HBoV infections were more often detected in URTI or bronchitis cases than in pneumonia and/or bronchiolitis (P_o=0.0123). In the 2nd season all 5 detected HBoV infections manifested as URTI or bronchitis.

DISCUSSION

The surveillance system of viral respiratory infectious diseases in Poland is limited to influenza. Problem of other than influenza viral respiratory infections, is still unrecognised, especially among young children. In some other countries confirmed cases of viral respiratory infections are reported and analysed. There are some differences in panel of monitored viral activity: in Canada – infections of influenza viruses (A and B), RSV, HPIV type 1-4, HMPV, HRV, HAdV and coronaviruses; in the USA: influenza viruses, RSV, HPIV, HAdV; in England and Wales the activity of influenza viruses, HAdV, RSV, HPIV and HRV is monitored (2-4).

The aim of our study was to determine the occurrence other than influenza virus infections among children hospitalized in one hospital in Warsaw during 31 months of the study (2008-2011) and to analyse the seasonality of the identified viruses. The high percentage of detected viruses in collected samples (72.7% positive) was an effect of a wide panel of examined viruses (RSV, HMPV, HEV/HRV, HPIV type 1,2,3 and HAdV, HBoV), and a good cooperation with the clinicians. The panel of being detected viruses didn't

include influenza viruses in our study because of age of patients. The number of influenza cases among children below 5 usually was lower in comparison with RSV or other respiratory viruses (11-15). Moreover, during A/H1N1v.2009 pandemic, tests for influenza were usually done by GPs prior to admission to the hospital.

Generally, in our study, RSV was the main aetiological agent of acute viral infections and was the main reason for children's hospitalization, but mainly those patients with LRTI. Among patients with diagnosed RSV infections only 1.2% have got symptoms of URTI. High prevalence of RSV determined among acute viral lower respiratory tract infections was related to examined group: the majority of patients were below 6 months of life (including preterm infants), followed by patients below 3 years old and only 4% of all of them were 3-5 y.o. In such age-groups the prevalence of RSV is usually very high. Moreover, the most severe LRTIs (bronchiolitis, pneumobronchiolitis, pneumonia) were also caused by RSV, especially in RSV epidemic season – as it was described by other authors (9,16). However, as it was found in our study, severe LRTI cases might have been caused by other, than RSV, viruses, especially in summer months when pneumonia was mainly caused by HEV/HRV. Our results are similar to data collected during many years of monitoring of viral activity in USA, Canada and the UK (2-4). In those countries, spring and summer time is usually connected with parainfluenza viruses (mainly type 3), Enteroviruses, Rhinoviruses, Adenoviruses and HMPV infections. Furthermore, in our studies, Enteroviruses, HMPV, HPIV and HAdV infections were detected in summer/fall time, however some single RSV cases were also identified. In opposite, human bocavirus infections, observed mainly in winter time, were rather connected with bronchitis or URTI than to pneumonia or bronchiolitis.

In our studies, the very high number of HMPV infections (21.2%) was observed in 2009. In other years, the frequency of HMPV was much lower. According to 2012 - 15th ESCV meeting data the increased number of HMPV cases in 2009 also was observed in other countries: England, Greece, Germany, Spain (13,17,18). The question is whether the increased frequency of HMPV infections across Europe in 2009 might have been caused by the increased number of paediatric consultations and hospitalization as a possible impact of "swine influenza" emergency, as it was observed in some countries (19). This thesis may explain our results indicating that patients with HMPV infection in 2009 were older than in other periods and that the main recognition in these patients (and only in this season) was acute URTI due to HMPV.

Usually, a peak of respiratory infections is observed in period between November - April every year in the European countries and other countries on the northern Hemisphere like the USA and Canada (2-4). In those months, the increased number of influenza viruses, RSV, HMPV, Rhinovirus, HPIV (mainly type 1), adenoviruses and other viruses infections are expected. Activity of some respiratory viruses might overlap in the winter-spring season, some viruses might be followed by another, and some viruses might be replaced by ones other. Similar epidemiological situation occurred every year, however, some differences are observed. Although, the activity of RSV and influenza viruses takes place at the same time, usually when one virus is predominant, the activity of the second one decreases. Such phenomenon was also described in the season 2009-2010, when A/H1N1v2009 appeared, one of the best examined season. In Italy few RSV infections were notified in November 2009, the number of cases increased in December with a peak in February and decrease in March 2010. At the same time, when the number of RSV infections increased - the number of identified cases of influenza (A/H1N1v2009) decreased (12). Similar viral activity was also observed in New York. When number of RSV infections increased in November 2009 (peak in January) number of influenza cases decreased, especially among babies and children (13). As for Central England very high peak of RSV cases was observed in January-February 2010 (14).

In our study, the beginning of the RSV epidemic season was different in examined years, and for example it was delayed in 2009-2010 in comparison with two other examined seasons. It might have been connected with high number of HMPV infections in the Fall of 2009 or with high activity of influenza virus (not examined), which were replaced by RSV cases. Another explanation of this phenomenon may be connected to biennial cycle of RSV outbreak, as it was described in Croatia. As result of monitoring of RSV activity during 12-years, it was found that RSV outbreaks occurred in two-year cycles, which were repeated every 23-25 months - after one large RSV epidemic season, the next one was smaller (20). Moreover, according to CDC data – the duration of RSV season may vary depending on the year and geographic region. In the USA the duration of RSV season 2010-2011 varied from 15 weeks (Kansas City) to 27 weeks (Florida) (3).

Even though, RSV is a predominant viral agent of respiratory infections in winter-spring season in children, the beginning of this season may differ from the occurrence of RSV infections and it should be taken into consideration the activity of other viruses. That was the reason why periods with increased number of all acute viral respiratory infections were separated. Duration of these periods with increased number of hospitalized children differed from 3 months in the first year of the study (XII.2008-II.2009), through 6 months – in the second (I.2010-V.2010) to 4 months – in third year

(XII.2010-III.2011). Variability of virus activity was observed in all periods: these with higher number of ARTI and those with lower number of patients. Also an unexpected increase of occurrence of particular viruses (like HBoV in 2008y., HMPV in 2009y. and HAdV in 2010y.) was found. Observed differentiation of clinical manifestations of LRTI was rather connected with the number of patients than with the kind of detected viruses. In opposite, the high frequency of URTI was rather connected to high activity of some viruses like HMPV and HBoV in the periods.

CONCLUSIONS

Observed variability of viruses activity might have been partially explained by temporary predomination of particular viruses in our region in different years. Relatively high prevalence of acute URTI or bronchitis caused by HBoV or HMPV in some periods indicated necessary of more detailed molecular and epidemiological studies. Also our results indicate the need for improved diagnostic capabilities of virological tests in acute upper and lower respiratory tract infections in children.

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