

# The Burden of Influenza, Respiratory Syncytial Virus and Human Metapneumovirus in Infants and Young Children in N. Greece, 2004-2013

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## Article Information

Received: 15 Sep 2015

Accepted: 15 Nov 2015

Plagiarism software: Turnitin

## Keywords:

Influenza,  
RSV,  
hMPV,  
Young Children



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## ABSTRACT

**Background:** According to the World Health Organization (WHO), acute respiratory infections are a leading cause of morbidity and mortality in infants and children worldwide. The aetiology of many of these respiratory infections remains unknown, highlighting the potential role of unrecognized pathogens. The aim of the present study is to determine the contribution of hMPV, RSV and influenza virus infections to acute respiratory infections in children < 6 years old, during 2004-2013 in northern Greece. **Materials and Methods:** During 2004-2013, a total of 949 pharyngeal swabs were collected from patients younger than 6 years old, who were presented as Influenza-Like Illness (ILI) or other respiratory infections. The clinical specimens were divided into three age groups (0-6 months-119 specimens, 7 months-2 years-377 specimens, 3-6 years-453 specimens). A real-time one-step RT-PCR protocol with specific primers and probes for matrix protein and haemagglutinin genes was used in order to type and subtype influenza A and B viruses. A multiplex real-time one-step RT-PCR was used to detect hMPV and RSV in the extracted RNA. **Results:** Influenza viruses were detected in 343 out of 949 specimens, RSV in 82 and hMPV in 50 specimens. **Conclusion:** Our results show the significant role of these pathogens in childhood respiratory disease. They require constant medical attention as they represent a substantial health care burden among inpatients and outpatients.

According to the World Health Organization (WHO), acute respiratory infections are a leading cause of morbidity and mortality in infants and children worldwide.<sup>1</sup> The aetiology of many of these respiratory infections remains unknown, highlighting the potential role of unrecognized pathogens.

Influenza viruses are often responsible for acute respiratory infections (ARI), severe acute respiratory infections (SARI) and the subsequent hospitalization among infants and children. In Greece, the influenza epidemic season starts late in December and lasts until early in April, peaking during January and February. On the other hand, Respiratory Syncytial Virus (RSV)

is an important cause of SARI, lower respiratory track infections and bronchiolitis among children younger than 3 years old, especially infants. Moreover, mortality data demonstrate that RSV is the third leading cause of fatal lower respiratory infections in childhood, following pneumococcal pneumonia and Haemophilus influenzae b.<sup>2</sup>

In 2001, van den Hoogen et al., isolated a previously unknown viral pathogen from children and adults with acute respiratory infection in the Netherlands. The virus was classified as a new member of the genus of Metapneumovirus and was assigned the provisional name of human Metapneumovirus (hMPV).<sup>3</sup> Many investigators have since described patients with acute respiratory infection caused by hMPV, while many studies demonstrated that RSV and hMPV have a similar spectrum of disease.<sup>4</sup>

The aim of the present study is to determine the contribution of hMPV, RSV and influenza virus infections to acute respiratory infections in infants and

## Access this article online

Website:	Quick Response code
www.actamedicainternational.com	
DOI: 10.5530/ami.2016.1.32	

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children younger than 6 years old, during the past decade in northern Greece.

During 2004-2013, a total of 949 pharyngeal swabs were collected from patients younger than 6 years old (445 males and 504 females), who were presented as Influenza-Like Illness (ILI) or other respiratory infections. The patient median age was 3.2 years. The clinical specimens which were collected by the National Influenza Centre for Northern Greece were divided into three age groups (0-6 months-119 specimens, 7 months-2 years-377 specimens, 3-6 years-453 specimens). (Table 1). The pharyngeal swabs were collected within three days from the onset of symptoms and they were transported to the laboratory in viral transport medium within 24 hours. Every specimen was accompanied by a Standard Form, presenting the necessary demographic data, the date of specimen collection, detailed clinical signs and symptoms and the medical history of each patient.

RNA extraction was performed using the Qiagen Viral RNA mini kit, following the manufacturer's instructions (Qiagen, Hilden, Germany). A real-time one-step RT-PCR protocol with specific primers and probes for matrix protein and haemagglutinin genes was used in order to type and subtype influenza A and B viruses.<sup>5</sup> In parallel, a multiplex real-time one-step RT-PCR was used to detect hMPV and RSV in the extracted RNA. Statistical analysis of the results was performed with SPSS (version 16.0, SPSS Inc., Chicago, Illinois), by means of a chi-square test with two degrees of freedom.

**Table 1: Age distribution of cases and confirmed influenza virus, RSV and hMPV infections in North Greece, 2004-2013**

Age groups	Cases (n)	Influenza positive (n)	RSV positive (n)	h-MPV positive (n)
0-6 months	119	31	14	2
7 months-2 years	377	131	48	12
3-6 years	453	181	20	36
Total	949	343	82	50

**Table 2: Seasonal distribution of cases and confirmed influenza viruses, RSV and hMPV infections**

Influenza season	No. of samples	Influenza positive	AH3N2	AH1N1 seasonal	AH1N1 pandemic	B	RSV positive	hMPV positive
2004-2005	72	28	10	16	-	2	10	-
2005-2006	38	7	1	-	-	6	7	4
2006-2007	50	14	10	-	-	4	5	4
2007-2008	25	13	-	10	-	3	-	4
2008-2009	100	38	24	1	-	13	29	3
2009-2010	332	97	-	-	96	1	16	25
2010-2011	255	114	-	-	102	12	4	1
2011-2012	55	28	12	-	-	16	7	8
2012-2013	22	4	-	-	3	1	4	1
Total	949	343	57	27	201	58	82	50

According to our results, influenza viruses were detected in 343 specimens (36.14%), RSV in 82 specimens (8.64%) and hMPV in 50 specimens (5.26%) (Table 2).

During the first four periods of study, 2004-2008, none of the young patients developed pneumonia, bronchiolitis or was hospitalized. Co-infections of hMPV and influenza viruses were observed in three cases; in detail, one case of hMPV and B influenza virus infection was observed in 2004-2005 and two cases of hMPV and seasonal A(H1N1) were observed in 2007-2008.

During the following 2008-2010 seasons, the most common clinical findings in infected young patients were fever, rhinorrhoea and wheeziness. Although during this period 22 patients developed pneumonia and were therefore hospitalized, only two of them were positive for influenza viruses, five were RSV positive, while the majority of them were hMPV positive. Co-infections of influenza virus and RSV were observed in seven cases, influenza virus with hMPV in two cases, while co-infection with RSV and hMPV were identified in four cases. There was also one case of a 5 year old boy with a triple co-infection between RSV, influenza B virus and influenza A(H3N2) virus.

Interestingly, during the post pandemic period 2010-2011, an increased number of young patients developed pneumonia and were therefore hospitalized. Fifteen hospitalized patients were found positive for influenza and one for RSV. Remarkably, during the post pandemic period 2010-2011, only 0.15% and 0.03% of the young patients were positive for RSV and hMPV infections respectively. Co-infection was found in only one case between hMPV and A(H1N1)pdm09.

During the last two periods of study, 2011-2012 and 2012-2013, co-infections were observed only during 2011-2012; two cases with influenza and hMPV, one with RSV and hMPV and one case with influenza B and RSV. Five children were hospitalized during this period, three

of them due to influenza infection and the remaining due to RSV infection.

Overall, co-infections of RSV and hMPV were observed in 5 cases, RSV and influenza viruses in 9 cases and hMPV with influenza viruses in 8 cases. No statistically significant difference was observed between co-infections of RSV and hMPV, RSV and influenza virus, or influenza virus and hMPV ( $p=0,412$ ). Interestingly, co-infections with hMPV occurred with all the subtypes of the influenza viruses detected (A(H3), A(H1) and B). Co-infections have been reported to occur in many studies, at a rate of 4-25% in cases with hMPV, RSV, influenza and various other viruses.<sup>4</sup> As in previous studies, this report confirms that co-infections are possible, but the role of hMPV is still unknown, as its contribution as a co pathogen is still largely unclear.<sup>6</sup> Moreover, in agreement with the majority of the studies, there was no evidence that patients co-infected with hMPV and influenza viruses suffered from more severe disease, although Semple *et al.* recently suggested that dual infection with hMPV and RSV is associated with increased severity as judged by mechanical ventilation and intensive care unit admission.<sup>7</sup>

According to the age distribution, the majority ( $n=453, 47,7\%$ ) of the young patients were 3-6 years old. The incidence of infections and specifically infections attributed to influenza was higher in the age group of 3-6 years old, as 181 out of 343 influenza infected patients (52,7%) belonged to this group, and this was a statistically significant difference ( $p=0.0148$ ). In addition, 36 out of 50 hMPV infected patients (72%) belonged to the age group of 3-6 years old as well ( $p<0.001$ ). In contrast, the incidence of RSV infection was higher in the age groups 7 months-2 years (58,5%), ( $p<0.001$ ), while in the age group of 0-6 months, RSV infections were only detected in 17,07% of the cases. This is an interesting observation, as it demonstrates that RSV is associated with infants mostly in terms of the severity of disease, than its frequency. Notably, no statistically significant difference was observed between sex and the type of the detected pathogen.

The clinical pattern of hMPV more closely resembles that of RSV than that of influenza SARI, yet the differences in age, radiographic findings and clinical diagnosis suggest that hMPV pathogenesis may differ from that of RSV. hMPV is more often a cause of pneumonia, while RSV a cause of bronchiolitis. Moreover, hMPV patients were on average older than RSV patients.

On the other hand, hospitalization rates in our study demonstrated that 20 out of 343 (5.8%) young patients infected from influenza viruses developed pneumonia and were therefore hospitalized. The majority of these hospitalizations occurred mainly in the post pandemic

season. This can be partially explained by the fact that clinicians altered their specimen collection policy and mostly focused to ALRI cases during the above period. RSV contributed to hospitalization at a rate of 9.7% (8 out of 82 cases), mainly during the pandemic season, while 16% (8 out of 50 cases) of hMPV infections lead to hospitalization ( $p=0.0286$ ). Interestingly, a burst of hMPV infections occurred during the influenza pandemic period and all of the hMPV associated hospitalizations were reported during this period. However, Edwards *et al.* recently published a study in the *New England Journal of Medicine*, where it is suggested that the potential inpatient burden of disease associated with hMPV is similar to that associated with other common respiratory viruses.<sup>6</sup>

Regarding the hMPV burden, previous studies are in agreement with our results, that it accounts for more than 4% of all respiratory infections in children during the winter season, while on an annual level, it accounts for 1-2% of all respiratory infections.<sup>8</sup> This estimate also agrees with the results of a recent 2-year study in which the prevalence of hMPV ranged from 1% to 5% of all upper respiratory infections in a given year in children <5 years of age.<sup>9</sup>

In the majority of the periods of study, hMPV and RSV infections both culminated in February-March, while influenza infections reached a peak in December-January. Interestingly, during the pandemic period, despite the fact that the surveillance for 2009-2010 season started in May, instead of December 2009, the seasonal distribution of hMPV and RSV remained the same as the previous years and did not follow the pandemic influenza pattern. That year, most hMPV and RSV cases appeared in February, while the majority of influenza cases were observed in November of that season. According to previous studies, the presence of hMPV and RSV infection is observed from the beginning to the end of each influenza season peaking during winter-spring.<sup>10</sup>

In conclusion, our results show the significant role of these pathogens - influenza viruses, hMPV and RSV - in childhood respiratory disease. They require constant medical attention as they represent a substantial health care burden among inpatients and outpatients.

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**How to cite this article:** Gioula G, Melidou A, Exindari M, Papalexis P, Xanthis D, Malisiovas N. The burden of influenza, respiratory syncytial virus and human metapneumovirus in infants and young children in N. Greece, 2004-2013. *Acta Medica International.* 2016;3(1):154-157.

**Source of Support:** Nil, **Conflict of Interest:** None declared.