Contribution of human metapneumovirus to influenza-like infections in North Greece, 2005-2008

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Citation style for this article:
Citation style for this article: Gioula G, Chatzidimitriou D, Melidou A, Exindari M, Kyriazopoulou-Dalaina V. Contribution of human metapneumovirus to influenza-like infections in North Greece, 2005-2008. Euro Surveill. 2010;15(9):pii=19499. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19499

This article has been published on 4 March 2010

Following its detection in 2001, human metapneumovirus (hMPV) has repeatedly been reported as a respiratory pathogen, especially in children. This study was aimed at determining the proportion of hMPV infections in patients with influenza-like illness (ILI) during the three influenza seasons 2005-6, 2006-7, 2007-8 in northern Greece. We collected 380 nasopharyngeal swabs or aspirates from ILI patients during the winter seasons 2005-2008 and examined them for influenza viruses and hMPV by one-step real time RT-PCR and nested RT-PCR. Influenza viruses were detected in 151 of the 380 specimens (39.7%) and hMPV in 23 of them (6.05%). Co-infections with hMPV and influenza viruses were observed in seven cases. The majority of the ILI patients (60.5%) were 0-18 years old. However, the incidence of influenza was slightly higher in the age group of 19-60-year-olds, while the incidence of hMPV infections was higher in the age group of 0-18-year-olds. We conclude that hMPV plays an important role as a contributor in influenza-like infections, especially in children. It circulates in the community during influenza seasons and its clinical appearance can be confused with influenza symptoms. However, further research is needed to elucidate the quantitative and qualitative importance of hMPV infections.

Introduction

In 2001, van den Hoogen *et al.* isolated in cultures of tertiary monkey kidney cells a novel paramyxovirus and identified it by random arbitrary polymerase chain reaction (PCR) [1]. The virus was classified tentatively as a new member of the genus *Metapneumovirus* and assigned the provisional name of human metapneumovirus (hMPV). Detection of viral gene sequences by reverse transcription-PCR (RT-PCR) directly from respiratory secretions made it possible to demonstrate rapidly that the virus was occurring worldwide and in all age groups [2].

Influenza viruses, which also circulate in the winter season, are the leading aetiological agents of respiratory tract illness in young adults [3]. In Greece, the influenza season starts in late December and lasts until early April, peaking in January and February [4-6], and the seasonal surveillance programme on cases of influenza-like illness (ILI) starts earlier in November. The National Influenza Centre for North Greece, one of two National Influenza Centres in Greece, examines clinical samples from ILI patients, including infants and children, every year. The sentinel surveillance system has been responsible for ILI surveillance in Greece since December 1999. The collected data are reported by e-mail to the Hellenic Centre for Diseases Control and Prevention.

The aim of this study was to determine the contribution of hMPV infections to ILI during the three influenza seasons 2005-6, 2006-7 and 2007-8 in North Greece.

Materials and methods

During the three influenza seasons 2005-2008, the National Influenza Centre for North Greece collected 380 nasopharyngeal swabs or aspirates from ILI patients, 214 male and 166 female patients between one month and 85 years of age (mean age 19.94 years). Of these specimens, 158 were collected during the 2005-6 local influenza season, 129 in the season 2006-7) and 93 in the season 2007-8 (Table 1). The ILI patients were further divided into five age groups (0-5 years, 6-10 years, 11-18 years, 19-60 years and >60 years) (Table 2).

The specimens were collected within three days of the onset of symptoms by general practitioners and paediatricians participating in the Greek sentinel influenza surveillance programme, and they were transported to the laboratory in sucrose phosphate medium within 24 hours at a temperature of 4°C. All specimens were accompanied by a sentinel standard form with information on age, sex, date of onset, date of specimen collection, place of residence and clinical features of each patient. All cases reported in this study met the Greek case definition for ILI, which comprises the following symptoms: acute onset, high fever (>38°C), cough, muscle ache and/or headache and malaise. These data were extracted from the summaries prepared by the reporting physicians. As the purpose of this study was

to test only the ILI cases, only patients who met these criteria were included.

Virus detection and typing

Following RNA extraction from clinical specimens, one-step real time RT-PCR for the detection of influenza A and B viruses was performed, using primers specific for matrix protein and nucleoprotein genes of influenza A and B [7]. To subtype the influenza A viruses, a nested RT-PCR was performed targeting the haemagglutinin gene using specific primers provided by the Influcheck kit (Euroclone, Italy).

For the detection of hMPV in clinical specimens, a one-step real-time RT-PCR method was used following the protocol by Bonroy *et al*. [8].

Statistical analysis

Statistical analysis of the results was performed with SPSS (version 11.0). For the different age groups, the median age and the infection rates were estimated by means of descriptive statistics. The chi-square test was used to compare the infections rates for both influenza and hMPV infection among different age groups.

Results

The median age of the 380 ILI patients was 19.94 years. They had mostly upper respiratory infections and none of them was hospitalised. Influenza viruses were detected in 151 specimens (39.7%) and hMPV in 23 (6.05%) (Table 1). Co-infections with hMPV and influenza virus were observed in seven cases.

In the 2005-6 season (158 ILI cases), 54 (34.1%) of the specimens were positive for influenza viruses (median age of the patients 16.6 years). The predominant type during that season was influenza B (44 of 54, 81.4%). hMPV was the causative agent of ILI in eight cases (5.06% with median age of the patients 8.8 years). Co-infections with influenza virus and hMPV were identified in three of the eight cases [two with influenza B and one with influenza A(H3)].

During the 2006-7 season (129 ILI cases), 51 (39.5%) of the specimens were positive for influenza or hMPV infection. Influenza A(H3) was the predominant subtype (88.2%), while influenza B contributed to 11.7% of the infections. hMPV was detected in eight cases (6.2%) and two of them were co-infections, both with influenza A(H3) virus. The median age of the infected patients during this season was 24.1 years for influenza and 10.5 years for hMPV infection. Influenza A(H1) was not isolated in either of the winters 2005-6 or 2006-7.

In the 2007-8 influenza season, 46 of 93 specimens (49.4%) were positive for influenza viruses. Influenza A(H1) was the predominant subtype (67.3%), while influenza B virus was detected mostly at the end of the season at a rate of 32.6%. Seven specimens were identified as positive for hMPV (7.53%) (Table 1). Viral co-infections between hMPV and influenza A(H1) virus were observed in two of the seven positive cases. The median age of influenza- and hMPV-infected patients during this season was 22.3 and 21.5 years,

According to the age distribution (Table 2), the majority (n=230, 60.5%) of the ILI patients were 0-18 years old. Only 21 of the 380 patients examined were older than 60 years (5.5%). The incidence of influenza infection was slightly higher in the age group of 19-60-year-olds, as 58 of the 129 influenza-infected

Table 1

Detection of influenza virus and human metapneumovirus in ILI cases, North Greece, influenza seasons 2005-2008 (n=380)

Influenza season	ILI cases (n)	Influenza-positive (n)	Influenza A(H3) (n)	Influenza A(H1) (n)	Influenza B (n)	hMPV (n)
2005-6	158	54	10	0	44	8
2006-7	129	51	45	0	6	8
2007-8	93	46	0	31	15	7
Total for 2005-2008	**380 (100%)**	**151 (39.7%)**	**55**	**31**	**65**	**23 (6.05%)**

hMPV: human metapneumovirus; ILI: influenza-like illness.

Table 2

Age distribution of ILI cases and confirmed influenza virus and hMPV infections, North Greece, influenza seasons 2005-2008 (n=380)

Age group (years)	ILI cases (n)	Influenza-positive (n)	hMPV-positive (n)
0-5	94	23	12
7-10	82	26	4
11-18	55	37	1
19-60	128	58	5
>60	21	7	1
Total	**380**	**151**	**23**

hMPV: human metapneumovirus; ILI: influenza-like illness.

patients (44.9%) belonged to this age group. The median age of influenza- and hMPV-infected patients was 20.9 years and 13.6 years, respectively. The incidence of hMPV infection was highest in the age group of 0-18-year-olds, with 17 of the 23 hMPV-infected patients (73.9%) in this age group. Finally, 16 of the 23 hMPV-infected patients (69.5%) were under 10 years old.

Statistical analysis of the results demonstrated that there was a statistically significant difference between the age groups with respect to influenza and hMPV infection in the 2005-6 season. Younger patients up to the age of 18 years were more likely to be infected by these two viruses (p=0.03 for influenza and p=0.005 for hMPV). On the contrary, there was no statistically significant difference in the following influenza season 2006-7 in the ages of the patients with respect to hMPV infection (p=0.472), while there was a statistically significant difference between the age groups with respect to influenza infection, with older patients (19-60 years old) more likely to get influenza in that season (p=0.026). No statistically significant difference between the age groups was found for influenza or hMPV infections in the third period 2007-8 (p= 0.161 for influenza and p=0.247 for hMPV). We did not observe any statistically significant correlation of between sex and the probability of being infected by either hMPV or influenza virus (p=0.500 for influenza and p=0.061 for hMPV).

We further analysed the clinical severity of the 23 cases infected and co-infected with hMPV. The most common clinical findings in patients infected only with hMPV were fever lasting on average three days (n=21), cough (n=18), rhinorrhoea (n=17) and wheezing (n=15). The duration of the fever was ascertained during follow-up by the physicians, done for all 23 hMPV patients to monitor symptoms that developed later. Chest radiographs were obtained for 10 patients. Abnormal findings such as peribronchial cuffing, prominent hilum and focal infiltrates were noted. It should be noted that the chest x-rays were taken in adults with severe symptoms, as radiological examination is unusual for children with common respiratory infections as ILI in most European countries. Patients co-infected with hMPV and influenza viruses had almost the same symptoms as the ones who were infected only with hMPV.

Despite the fact that none of the patients was hospitalised, four children with hMPV and six with influenza virus infection required supplemental oxygen as a preventive measure, according to the physicians attending to them at the outpatient clinic. Seven hMPV-infected children were treated with antibiotics for a median of five days (range: 1-14 days). The most frequently reported symptoms of the eight patients aged over 60 years (seven with influenza and one with hMPV infection) were mostly high fever over 38.5°C (n=8), muscle ache (n=8), dyspnoea (n=6) and sore throat (n=8). No hMPV-infected patient required mechanical ventilation or administration to the intensive care unit.

In the present study we screened only influenza virus and hMPV infections. Co-infections with respiratory syncytial virus (RSV) and other respiratory pathogens require additional studies.

Discussion

Human metapneumovirus is an emerging pathogen which has been associated with symptoms ranging from mild upper respiratory tract infections to severe pneumonia, exacerbation of asthma and chronic obstructive pulmonary disease [9-14]. Serological studies show that the virus has been circulating undetected in humans for at least 50 years [1]. It is thus an important pathogen and it is essential to obtain a better understanding of its contribution to acute respiratory infections.

As the virus can cause clinical signs and symptoms that resemble influenza [15], the aim of the present study was to determine the contribution of hMPV to ILI during the three influenza seasons 2005-8 in North Greece. According to our results, hMPV contributed to ILI at a rate of 6.05%, while influenza viruses were the main cause of the disease (39.7%). Although the overall prevalence of hMPV was low, it played an important role as a contributor to ILI, especially in children, as the majority of the hMPV-infected patients (69.5%) were under 10 years old.

In the influenza season 2005-6, 5.06% of ILI patients were hMPV-positive, in 2006-7, it was 6.2% and 6.05% in 2007-8. In a similar study conducted in Japan from 2002 to 2004, hMPV positivity rates in patients with ILI were 5.7% in 2002-3 and 5.2% in 2003-4 [16], while in another study in Finland from 2000 to 2002, hMPV was responsible for 7% of all respiratory infections in children, even though influenza was circulating in the community at the same time [17].

Co-infections of hMPV with RSV, influenza and various other viruses have been reported in many studies, at a rate of 4-70% [17]. In the present study, hMPV was detected in 23 ILI cases, seven of which were co-infections with influenza viruses. Co-infections with hMPV occurred with all the subtypes of the influenza viruses detected (influenza A(H3), A(H1) and B). As in previous studies, this report confirms that co-infections are possible, but the clinical implications of hMPV in these cases is still unknown, as so little is known about its contribution as a co-pathogen [13,14,19]. In agreement with the majority of studies, there was no evidence that patients co-infected with hMPV and influenza viruses had more severe disease, although Semple *et al*. have recently suggested that dual infection by hMPV and RSV is associated with increased severity as judged by mechanical ventilation and intensive care unit admission [20].

Our findings demonstrate that the effect of hMPV is greatest in children, as the median age of hMPV-infected patients was 13.6 years during the three investigated influenza seasons. Sixteen of the 23 hMPV-infected patients (69.5%) were under 10 years old and about 50% of them belonged to the age group 0-5 years. According to previous studies, hMPV accounts for more than 4% of all respiratory infections in children during the winter season and for 1-2% of all respiratory infections annually [16]. This estimate agrees with the results of a recent 20-year study in which the prevalence of hMPV ranged from 1% to 5% of all upper respiratory infections in a given year in children under the age of five years [21].

In the present study, younger patients (0-18 years old) were more likely to be infected by influenza virus or hMPV in the 2005-6 season, in contrast to 2006-7, when older patients (19-60 years old) had a higher probability of getting influenza. According to information from the former European Influenza Surveillance Scheme (EISS), the highest consultation rates for ILI during the 2006-7 winter season were generally observed among children aged 0-4 years and 5-14 years. However, in some countries the population under surveillance was skewed to the younger ages (partly due to a high proportion of paediatricians participating in EISS) and/or older ages [22].

hMPV was detected from the beginning to the end of each influenza season examined, and was circulating during the whole period. These findings are consistent with previous studies that demonstrate the seasonal distribution of hMPV infections, which resembles that of influenza with recurrent epidemics during the winter [12,13]. In conclusion, our results show that hMPV is an emerging cause of acute respiratory infection in ILI patients and may have a significant clinical impact, especially in children. However, further research is needed to elucidate the quantitative and qualitative importance of hMPV infection, its seasonal distribution, the groups at risk of severe complications, and strategies for its diagnosis, treatment and prevention.

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