Viral causes of Influenza-Like Illness: Insights from a study during the winters 2004-2007

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Table 1.

Number of positive samples, by specific virus and month of diagnosis (the percentage is calculated from total number in a column)

	November (n=28)	December[a] (n=151)	January[b] (n=123)	February[c] (n=208)	March[d] (n=61)	April (n=9)
	n. (%)	n. (%)	n. (%)	n. (%)	n. (%)	n. (%)
Influenza virus	1 (4)	12 (8)	37 (30)	65 (31)	13 (21)	4 (44)
Adenovirus	0 (0)	22 (15)	11 (9)	26 (13)	4 (6)	0 (0)
PIV	5 (18)	9 (6)	7 (6)	12 (6)	2 (3)	0 (0)
RSV	0 (0)	5 (3)	2 (2)	3 (1)	0 (0)	0 (0)
Other/nd	22 (79)	106 (70)	70 (57)	112 (55)	45 (74)	5 (56)

NOTE. [a] 3 coinfections (1 RSV + Adenovirus; 2 Adenovirus + PIV)
[b] 4 coinfections (1 Influenzavirus + Adenovirus; 3 Influenzavirus + PIV)
[c] 10 coinfections (8 Influenzavirus + Adenovirus; 1 RSV + Adenovirus;1 Influenzavirus + PIV)
[d] 3 coinfections (3 Influenzavirus + Adenovirus)

Table 2.

Number of positive samples, by specific virus and age class (the percentage is calculated from total number in a row)

	FLU	ADENO	PIV	RSV	OTHER/ND
Age (years)	N (**%**)	N (%)	N (%)	N (%)	N (%)
0 – 2 (63)	20 (32)	13 (21)	3 (5)	3 (5)	29 (46)
3 – 13 (161)	42 (26)	19 (12)	10 (6)	3 (2)	94 (58)
14 – 64 (319)	69 (22)	27 (8)	20 (6)	4 (1)	207 (65)
≥65 (37)	1 (3)	4 (11)	2 (5)	0 (0)	30 (81)

Table 3.

Number of samples, by specific virus and sign and symptom.

Sign/symptom	Influenza (n.116)		Adenovirus (n.47)		PIV (n.29)		RSV (n.8)		Other/nd (n.360)	
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
Sore throat	76	(66)	26	(55)	20	(69)	3	(38)	237	(66)
Nasal congestion	70	(60)	21	(45)	14	(48)	4	(50)	179	(48)
Muscle pain	63	(54)	10	(21)	5	(17)	1	(12)	134	(37)
Headache	53	(46)	15	(32)	13	(45)	1	(12)	141	(39)
Dry cough	57	(50)	21	(45)	12	(41)	2	(25)	183	(51)
Productive cough	41	(36)	17	(36)	6	(21)	7	(87)	110	(31)
Shivers	41	(36)	10	(21)	8	(28)	1	(12)	130	(36)
Joint pain	42	(36)	10	(21)	3	(10)	1	(12)	91	(25)
Retrosternal pain	28	(24)	7	(15)	5	(17)	2	(25)	65	(18)
Sweating	23	(20)	4	(8)	5	(17)	3	(37)	72	(20)
Abdominal pain	14	(12)	9	(19)	3	(10)	0	(0)	31	(9)
Dyspnea	12	(10)	4	(8)	1	(3)	3	(37)	46	(13)
Nausea	12	(10)	3	(6)	4	(14)	0	(0)	43	(12)
Vomiting	8	(7)	5	(11)	3	(10)	0	(0)	34	(9)
Diarrhoea	7	(6)	7	(15)	3	(10)	1	(12)	22	(6)
Shortness of breath	0	(0)	1	(2)	0	(0)	0	(0)	1	(0)

Table 4.

Crude and adjusted OR (and 95% CI) of Influenza, according to gender, age, month of diagnosis and symptoms.

Variables	OR	95% CI	Adj for variables *	95% CI
females vs. males	0.82	0.56 1.21	-----	
age x 10 years	0.89	0.81 0.98	0.83	0.74 0.93
Month of diagnosis				
December (ref.)	1.00	---	1.00	---
Jan	6.39	3.23 12.64	7.11	3.53 14.33
Feb	4.74	2.42 9.27	5.32	2.65 10.64
March	3.39	1.45 7.97	3.75	1.55 9.06
April	9.40	2.22 39.71	8.25	1.90 35.78
Nov	0.33	0.04 2.59	0.38	0.05 3.08
Symptoms				
Dry cough	0.86	0.58 1.26	---	
Productive cough	1.24	0.83 1.87	---	
Dyspnea	0.80	0.42 1.51	---	
Sore throat	1.07	0.71 1.62	---	
Headache	1.18	0.80 1.75	---	
Shivers	1.10	0.73 1.65	---	
Sweating	1.01	0.62 1.66	---	
Retrosternal pain	1.29	0.80 2.09	---	
Nasal congestion	1.48	1.00 2.20	1.50	0.97 2.31
Muscle pain	1.97	1.33 2.91	2.00	1.25 3.20
Joint pain	1.82	1.20 2.76	1.81	1.09 3.01
Nausea	0.94	0.50 1.77	---	
Vomit	0.71	0.33 1.49	---	
Abdominal pain	1.30	0.71 2.39	---	
Diarrhoea	0.87	0.39 1.94	---	
Shortness of breath	---		---	
Earache	0.77	0.36 1.63	---	

with a p-value < 0.10 at univariate level.

Table 5.
Crude and adjusted OR (and 95% CI) of Adenovirus, according to gender, age, month of diagnosis and symptoms

Variables	OR	95% CI		Adj for variables *	95% CI	
females vs. males	0.84	0.50	1.41	-----		
age x 10 years	0.92	0.81	1.04	-----		
Month of diagnosis						
December (ref.)	1.00	---				
Jan	0.69	0.32	1.49			
Feb	1.20	0.63	2.30			
March	0.56	0.18	1.72			
April	---					
Nov	0.91	0.29	2.87			
Symptoms						
Dry cough	0.78	0.46	1.32	---		
Productive cough	1.12	0.65	1.95	---		
Dyspnea	0.63	0.24	1.64	---		
Sore throat	0.65	0.38	1.10	---		
Headache	0.64	0.36	1.12	---		
Shivers	0.63	0.35	1.15	---		
Sweating	0.34	0.13	0.87	0.41	0.16	1.06
Retrosternal pain	0.70	0.33	1.45	---		
Nasal congestion	0.78	0.46	1.32			
Muscle pain	0.40	0.21	0.75	0.43	0.22	0.82
Joint pain	1.01	0.56	1.83			
Nausea	0.52	0.18	1.47	---		
Vomit	0.88	0.34	2.31	---		
Abdominal pain	2.07	1.01	4.22	2.18	1.05	4.52
Diarrhoea	1.54	0.62	3.84	---		
Shortness of breath	8.32	0.51	134.73	---		
Earache	0.95	0.36	2.49	---		

* with a p-value < 0.10 at univariate level.

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Key words: Influenza-like illness, Respiratory viruses, Influenza, Adenovirus, Italy

Short running headline: Clinical predictors of influenza and Adenovirus associated influenza-like illness

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ABSTRACT

Limited information is available on the viral etiology of influenza-like illness in southern European countries, and it is still a matter of debate whether certain symptoms can be used to distinguish the specific viruses that cause influenza-like illness. The main objective of the present study was to identify the demographic and clinical predictors of influenza-like illness due to specific viruses. The study, which was observational in design, was conducted in Rome and Naples, Italy. Cases of influenza-like illness were defined as individuals with fever >37.5°C and at least one systemic and one respiratory symptom, recruited during the winters of 2004-2005, 2005-2006 and 2006-2007. Influenza and other respiratory viruses were identified using the polymerase chain reaction (PCR), carried out on throat swabs. Basic individual information was collected using a standard form. A total of 580 persons were included in the analysis. Viral pathogens were identified in fewer than 50% of the cases. Overall, 240 viruses were detected: 22.8% were positive for influenza viruses, 10.9% for adenoviruses, 6% for parainfluenza viruses, and 1.7% for respiratory syncytial virus. The month of diagnosis, and muscle and joint pain were associated with influenza virus, although the positive predictive value was low. Abdominal pain was associated with adenovirus infection. Although the positive predictive value of symptoms of influenza virus infection was low, especially during low activity periods, these findings may help diagnosis by clinicians.

INTRODUCTION

Respiratory infections are common in both adults and children, although the incidence of acute respiratory illness is highest in young children and decreases with increasing age [Badger et al.,1953]. Most acute respiratory infections are fairly mild, self-limiting, and confined to the upper respiratory tract, but severe illness may occur.

In industrialised countries, most of the respiratory infections which occur in winter have been attributed to viruses [Monto, 1995; Gwaltney, 2002]. However, the frequency of detection of specific viruses varies among studies, depending on the case-definition and diagnostic technique used, the type of specimen collected, the year of the study and the season [Badger et al., 1953]. The viruses detected most commonly have been rhinoviruses and influenza viruses, followed by parainfluenza viruses (PIV), respiratory syncytial virus (RSV), and adenoviruses [Badger et al.,1953; Heijnen et al., 1999]. The case-definition may affect the results: for example, for influenza-like illness, influenza viruses are detected most commonly, whereas rhinoviruses may rank first when a more generic definition of acute respiratory infection is used [van Gageldong-Lafeber et al., 2005]. Although high detection rates of RSV in influenza-like illness have also been reported [Lina et al.,1996], RSV infection is more likely to cause severe morbidity and higher mortality than influenza; for this reason, it is reported more commonly among hospitalized patients with severe acute lower respiratory infections, compared to persons in community-based studies [Fleming et al., 2005].

It is still a matter of debate whether certain symptoms can be used to distinguish among specific infections, although it is a common opinion that there are no symptoms specific for any viral infection. Although a few studies have attempted to identify signs or symptoms associated specifically with influenza virus [Peltola et al., 2005; Bolvin et al. 2000], no definitive conclusions have been drawn, and to the best of our knowledge, few studies have focused on influenza-like illness due to viruses other than influenza.

An observational study was undertaken with the following objectives: (i) to identify viruses responsible for influenza-like illness, (ii) to determine the proportion of cases attributable to each virus, and (iii) to identify the demographic and clinical predictors of influenza-like illness due to specific viruses.

MATERIALS AND METHODS

Study population and sample collection

The study design and methods have been described in detail elsewhere [Rezza et al. 2006]. Briefly, the study was conducted in Rome (winter seasons 2004-05, 2005-06 and 2006-07) and Naples (2005-06 and 2006-07), Italy, and in some peripheral areas of these cities. Eighteen general practitioners (GPs) from these areas participated, eight of whom were paediatricians. The physicians were asked to obtain a throat swab for all consecutive patients who, during the period November-April, presented with influenza-like illness within three days of the onset of symptoms; at the beginning of the winter season, each practitioner was provided with "Virocult swabs" (Medical Wire and Equipment, Corsham, UK). Influenza-like illness was defined as the presence of fever >37.5°C (axillary temperature) and at least another systemic symptom (i.e., headache, malaise, myalgia, chills or sweating, retrosternal pain, or asthenia) and one respiratory symptom (i.e., cough, sore throat, nasal congestion or runny nose). The minimum temperature for defining fever (>37.5°C) was slightly lower than that used by the Italian Ministry of Health for influenza-like illness surveillance (38°C) [Gabutti et al., 2004], so as to include persons with a milder fever.

Information on gender, age, clinical symptoms, and vaccination history was obtained for each participant. The clinical samples were sent to the laboratory for examination. Verbal informed consent was obtained from all of the participants. Because this was an observational study, ethical committee approval was not required, although the study was conducted in accordance with the ethical requirements of the Italian Ministry of Health.

RNA and DNA Extraction and PCR amplification

At the laboratory, two separate aliquots of each clinical sample were prepared. To identify influenza A and B viruses and RSV types A and B, a multiplex RT-PCR assay was carried out. Viral RNAs were extracted either directly from clinical samples or from virus-infected MDCK culture fluid using an RNA extraction kit (RNeasy; Qiagen, Santa Clara, CA). cDNA synthesis and amplification were carried out as described elsewhere [Puzelli et al., 2004]. PCR amplification was performed using primers targeting specific regions within the following genes: (i) influenza A nucleoprotein and influenza A/H1- and A/H3-subtype haemagglutinins; and (ii) influenza B haemagglutinin and neuraminidase. The primers used in the PCR reactions are available from the authors upon request.

To identify other respiratory viruses, total DNA and RNA was extracted from a separate aliquot of the clinical sample using Ultrasens kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. To verify the acid nucleic extraction (DNA and RNA), the nucleic acid was amplified with the β-actin gene [Rezza et al., 2006; Strassburg et al., 1999]: all of the samples were positive. The samples were tested for the presence of influenza virus, adenovirus, RSV, and PIV types 1, 2, 3 and 4 [Heim et al., 2003; Templeton et al., 2004].

Statistical analysis

The distribution of the specific viral agents was evaluated first by month of diagnosis and age of the participant. The distribution of signs and symptoms was determined according to the etiology (i.e., influenza virus, adenovirus, RSV, PIV and other/unknown). Odds ratios (OR) and their 95% confidence intervals (95% CI) were calculated to determine the association between each variable (i.e., age, month of diagnosis, symptoms) and the most common viral infections at the univariate level. The variables which were found to be associated statistically at the univariate level were included in a multivariate model to test their independent association with the specific viral etiology (i.e., the model was used only for influenza, since only one variable was found to have

been associated with adenovirus infection at the univariate level). The positive predictive value (PPV) of specific symptoms for specific viral etiology was also calculated.

RESULTS

During the three winters considered in this study, 580 persons were recruited: 173 (29.8%) in 2004-05, 190 (32.8%) in 2005-06, and 217 (37.4%) in 2006-07. Of the participants, 299 (51.6%) were females and 281 (48.4%) males. The median age was 24 years (range: 0.5-88 years); 224 (38.6%) participants were of paediatric age (≤13 years), of whom 89 were less than 3 years old. Of the study participants, 561 (96.7%) were Italian. With regard to the study area, 409 (70.5%) participants were recruited in the province of Rome and 171 (29.5%) in Naples.

Of the samples collected, 220 were positive for at least one of the four viruses; of these, 20 were positive for two viruses. In the case of 360 samples, virus was not detected or the virus was not identified. The virus detected most frequently was influenza virus (n=132 samples), followed by adenovirus (n=63), PIV (n=35), and RSV (n=10).

One hundred and eighteen isolates were identified as influenza A and 14 as influenza B. Of the influenza A viruses, H3-subtype strains prevailed in the 2004-05 season, whereas H1-subtype strains prevailed in the 2005-06 season; during the 2006/07 season, H3 strains were again prevalent (57%). Of the parainfluenza viruses isolates, 27 were parainfluenza viruses type 3; 5 were type 2; 2 were type 4; and one was type 1.

The number of samples, by specific virus and month of diagnosis, is shown in Table 1. Influenza virus tended to be the most common virus from January to April, whereas PIV and adenovirus were the viruses detected most frequently in November and December, respectively. Of the 20 coinfected participants, most (n=12) were positive for both influenza virus and adenovirus; four were positive for influenza virus and PIV, two for adenovirus and PIV, and two for adenovirus and RSV.

Influenza virus was detected most frequently during two winter seasons (i.e., 2004-05 and 2006-07), whereas adenovirus was detected more frequently than influenza virus in 2005-06 (i.e., 21% vs. 18%) (data not shown).

The number of samples, by specific virus and age class of the participant, is shown in Table 2. With regard to influenza viruses, the proportion of positive samples was highest in the youngest age group (0-2 years) and tended to decrease with increasing age (chi square for trend, $p<0.01$). Children (≤13 years; the two youngest age groups) were more than 2 times more likely than adolescents and adults/elderly (the two oldest age groups) to be infected with influenza viruses (OR: 1.56, 95% CI: 1.04-2.36). Only one of the 13 participants ≥65 years of age was positive for influenza virus.

With regard to influenza vaccination, 118 (20.3%) of the 580 participants had been vaccinated; of these individuals, 23 (19.5%) were infected by influenza viruses, compared to 109 (23.6%) of the 462 individuals who had not been vaccinated (difference not statistically significant; OR: 0.78, 95% CI: 0.46-1.33).

The number of samples, by specific virus and sign and symptom, is shown in Table 3. Muscle pain ($p=0.028$) and joint pain ($p=0.046$) were reported more likely in persons infected with influenza viruses, compared to those infected with other viruses, and nausea ($p=0.045$) was reported less likely. The crude and adjusted ORs for variables potentially associated with positivity for influenza viruses are reported in Table 4. At the multivariate analysis, influenza viruses were more likely to be detected between January and April (compared to December), in younger age groups (i.e., <10 years, compared to older persons), and in participants with muscle or joint pain (compared to participants with other symptoms). However, none of the symptoms associated with influenza positivity had a high PPV: muscle pain had the highest PPV (42.9%) during the months of flu activity (i.e., January and February), whereas the combination of symptoms with the highest PPV in these two months was nasal congestion plus muscle pain (48.3%); the PPV of this combination was 12.9% in low influenza activity months (i.e., November and December). Given that some symptoms

were reported less frequently by participants younger than 10 years, such as muscle pain (18% among those less than 10 years old vs. 46% among those 10 years of age or older) and joint pain (9% vs. 35%), which could be due in part to the inability of very young children to describe subjective symptoms, the logistic analyses were repeated excluding persons younger than 10 years of age, and the results were confirmed (data not shown). Similarly, sweating was reported less frequently by persons younger than 10 years of age (9% vs. 23%).

The crude and adjusted ORs for variables associated potentially with positivity for adenovirus are reported in Table 5. Adenovirus positivity was more likely to be detected among those reporting abdominal pain, whereas there was no association with either the month of diagnosis or age (Table 5). When excluding participants younger than 10 years of age, the adjusted OR for abdominal pain remained of the same magnitude yet was no longer significant statistically (results not shown).

DISCUSSION

In this study, about 38% of the samples were positive for at least one virus, which is consistent with the results of other studies in which from 36-38% [Lina et al., 1999; Vabret et al., 2003] to 58% [van Gageldong-Lafeber et al., 2005] of the samples were positive. In a study of community-acquired respiratory infections, including *Mycoplasma pneumoniae* and *Clamydophila pneumoniae*, in addition to viruses, at least one potentially pathogenic microorganism was detected in 52% of the swabs [Heijnen et al., 1999].

As was found in other studies [Heijnen et al., 1999; Lina et al., 1996; Vabret et al., 2003], influenza was the virus detected most commonly, which could in part be due to the large epidemic of infection with influenza virus in 2004-05. The finding that influenza vaccination did not have a protective effect was probably related to viral drift [Ansaldi et al., 2005]. As expected, the overall results showed that the proportion of samples positive for influenza viruses was higher in young children than in adults; this is likely to be due either to higher vaccination coverage among older

adults or to the duration of viral shedding in young children, which is usually longer than in adults and characterized by larger virus load.

In the present study, RSV was detected rarely, which differs from the results of a study, in which it was almost as common as influenza virus, with the highest impact in the youngest age groups [Lina et al., 1996]. The rarity of RSV detection could be due to not only the low proportion of children but also to the fact that children admitted to hospital were not included.

In this study, an attempt was made to identify symptoms associated with influenza and adenovirus infections. However, it is known that clinical diagnosis of influenza is not easy. In a prospective study of children ≤13 years old, the overall sensitivity of clinical diagnosis of influenza was 38% and the PPV was 32% [Peltola et al., 2005]. The analysis of a study involving 5-12 year-old children enrolled in two trials showed that cough, headache, and fever ≥38.2°C predicted independently positivity to influenza virus; the PPV of combined fever and cough was 83%, whereas sore throat and myalgia were negative predictors. Myalgia was found to be associated independently with influenza only among younger children (1-4 year-olds) [Ohmit et al., 2006]. In another study of children (>6 years of age) and adults, cough and fever >38 were the only factors associated with a positive PCR for influenza [Bolvin et al., 2000].

In the present study, muscle and joint pain were associated with influenza, whereas nasal congestion was associated only marginally with the infection; the month of diagnosis (from January to April) and older age were also associated with influenza virus. However, the PPV of these symptoms remained low, even in the months with high influenza activity. Only abdominal pain was associated with adenovirus infection, whereas there was no association with either age or month of diagnosis.

Before drawing conclusions, the limits and bias of this study should be mentioned. Firstly, some viruses (rhinoviruses and metapneumoviruses) and bacteria (*M. pneumoniae* and *C. pneumoniae*) were not studied. In particular, the inclusion of rhinoviruses might increase greatly the frequency of virus detection, as indicated by studies reporting a higher proportion of these viruses

compared to influenza viruses [Badger et al., 1953]; it could also explain the relatively high proportion of unidentified causes. Nevertheless, the results of this study do not differ significantly from those of other studies. Secondly, the specific influenza type may produce some differences in clinical features. Rhinorrhea and sick appearance have been reported more frequently by children with influenza A, whereas myalgia is more common among those with influenza B [Peltola et al., 2003]. Symptoms such as muscle ache and headache, which may be characteristic of influenza in adults, are reported by a low proportion of children and cannot be communicated reliably by those <3 years of age. Thirdly, the potential occurrence of false-negative results due to the variable sensitivity of the laboratory techniques and to the type of clinical samples cannot be ruled out completely. Finally, the timing of collection of samples may have decreased the rate of detection, since some swabs were taken up to four days after the onset of symptoms (when viruses may have been cleared, at least in part, by the immune response). For this reason, the maximum sample delay, which was set at four days in the first year of the study, was reduced to three days in the last two years.

In conclusion, influenza virus was the virus identified most commonly, followed by adenoviruses, whereas cases attributable to other viruses were uncommon. The comparison between persons affected by the two different viral infections permitted the identification of some predictors of a specific viral disease. Although the surveillance of respiratory viruses associated with influenza-like illness is not sustainable, because of the high costs and lack of preventive measures, limited etiological surveys may provide useful information on the effect of specific viruses affecting human populations in the winter season and may possibly allow predictors of specific viral infections to be identified. The findings of this study may help clinicians to discriminate between influenza virus, adenovirus, and other infections; due to the low PPV of symptoms and other correlates of infection for influenza virus, the decision to use specific anti-influenza drugs should not be based only on the clinical diagnosis of influenza. Rapid laboratory tests might be used to

confirm influenza virus infection and facilitate decision-making when the use of antiviral drugs is considered.

ACUTE RESPIRATORY INFECTION (ARI) STUDY GROUP

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Footnote: The authors declare that they do not have any commercial or other associations that might pose a conflict of interest.

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