RESPIRATORY VIRUSES AND INFLUENZA-LIKE ILLNESS: A SURVEY IN THE AREA OF ROME, WINTER 2004-2005

G Rezza[1], C Valdarchi[1], S Puzelli[1], M Ciotti[2], F Farchi[1], C Fabiani[1], L Calzoletti[1], I Donatelli[1], CF Perno[2]

Limited information is available on the viral aetiology of influenza-like illness (ILI) in Southern European countries. Hereby we report the main findings of a survey conducted in the area of Rome during the 2004-2005 winter season.

ILI cases were defined as individuals with fever >37.5°C and at least one constitutional symptom and one respiratory symptom, recruited during the survey period. Influenza and other respiratory viruses were identified using polymerase chain reaction (PCR) on throat swabs. Basic individual information was collected through a standard form.

Of 173 ILI cases enrolled, 74 tested positive for one virus, and two tested positive for two viruses. Overall, 33.5% of the cases were positive for influenza viruses, 5.2% for adenoviruses, 3.5% for parainfluenza viruses, 1.7% for coronaviruses, and 1.2% for the respiratory syncitial virus. The proportion of influenza virus detection was higher in the 'high influenza activity' period. The distribution of viral agents varied across age groups, influenza viruses being more likely to be detected in younger patients.

Viral pathogens were identified in less than 50% of ILI cases occurred during a high activity influenza season. The detection of other than influenza viruses was sporadic, without evidence of large outbreaks due to specific agents.

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Introduction

Respiratory infections are common in both adults and children. Most of them are fairly mild, self-limiting, and confined to the upper respiratory tract, but severe illness may sometimes occur.

Most respiratory infections occurring during the winter in industrialised countries are attributable to viral agents [1, 2]. The incidence of acute respiratory illness is highest in young children and decreases with increasing age [3].

The frequency of detection of specific viral agents varies between different studies, depending on case definition, diagnostic techniques, and seasonality [1]. When all respiratory illnesses are considered, rhinoviruses and influenza viruses are the most represented agents, followed by parainfluenza viruses (PIV), respiratory syncytial virus (RSV), and adenoviruses [1,4]. However, the findings may differ depending on the case definition used: as far as influenza-like illness (ILI) is concerned, influenza viruses are most commonly detected, whereas rhinoviruses may rank first when a more generic definition of acute respiratory tract infection is used [5]. High detection rates of RSV in ILI have also been reported [6].

Most of the above mentioned studies have been conducted in the United States or in central or northern Europe, while limited information is available from the Mediterranean area. The objectives of the present study were: (i) to identify viruses responsible for ILI, (ii) to determine their proportion, and (iii) to identify virus-specific clinical syndromes in an Italian population during a winter season.

1. Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy
2. Department of Molecular Virology, Policlinico Tor Vergata, Rome, Italy

Material and methods

The survey was conducted in the area of Rome. Nine general practitioners, including two paediatricians, were recruited (seven in urban or suburban areas and two from rural villages in the province of Rome). At the beginning of November and January, each doctor was provided with 20 virocult swabs and was asked to enrol all patients fulfilling the recruitment criteria (that is, the case definition, and maximum time interval between onset of symptoms and sample collection). All patients with ILI, as defined by the presence of fever >37.5°C and at least one other symptom (headache, malaise, myalgia, chills or sweats, retrosternal pain, asthenia) and one respiratory symptom (cough, sore throat, nasal congestion or runny nose), between November 2004 and March 2005, were eligible for the study. Our case definition was different from that provided by the Italian Ministry of Health for ILI surveillance [7], so that we could include milder febrile cases. A throat swab was collected from patients who received home visits from their doctor within four days after the onset of symptoms.

Sample collection

Throat swabs were taken from individuals presenting with ILI, using 'Virocult swabs' (Medical Wire and Equipment, United Kingdom). Essential information (such as date of sample collection, patient's initials, sex, age, clinical symptoms, vaccination status) was collected for each specimen. On arrival in the laboratory, separate aliquots of each clinical samples were prepared and used for RT-PCR analysis.

RNA and DNA Extraction and RT-PCR

A multiplex RT-PCR was performed to identify influenza A or B viruses. In this case, viral RNA were extracted either directly from clinical samples or from virus-infected MDCK culture fluid using an RNA extraction kit (RNeasy; Qiagen, Santa Clara, California, USA). cDNA synthesis and amplification procedures were carried out as described elsewhere (8). PCR was performed using specific primers which amplified regions within the genes for: (i) the influenza A nucleoprotein and the influenza A/H1- and A/H3-subtype haemagglutinins; (ii) the influenza B haemagglutinin and neuraminidase. Primers used in PCR reactions are available from the authors upon request.

In order to identify other respiratory viruses, total DNA and RNA was extracted from a separate aliquot of the clinical sample, by Ultrasens kit (Qiagen, Hilden, Germany), in accordand with the manufacturer's instructions. To verify the acid nucleic extraction (DNA and RNA), we amplified the nucleic acid with the b-actin gene (9): all the samples tested positive. Thus, the samples were screened for the presence of adenovirus, RSV, PIV type 1, 2, 3 and 4, enteroviruses, and coronaviruses, using primers sequences as reported [10-13].

Statistical analysis

The association between demographic variables or preventive measures (that is, vaccination) and specific viral infections was evaluated by using odds ratios (OR) and their 95% confidence intervals (95% CI). The statistical significance of other associations was assessed through the chi square test. Based on the number of ILI cases notified to FLU-ISS in the province of Rome, we identified a 'high' and a 'low/medium' influenza activity period, using a threshold

of 850 cases, which was about 60% of the maximum weekly number of cases (1436 cases reported during week 5). The distribution of specific viruses in the two periods was then compared. With regard to the association between each symptom and specific viral infections (that is, influenza versus other viruses), the Bonferroni correction was used to test the statistical significance of the associations, in order to minimise the risk of a Type I error in the presence of multiple outcome measures of importance [14].

Results

Overall, 173 patients with available samples were recruited during the study period. Of the participants, 96 (55.5%) were female and 77 (44.5%) male. The median age was 27 years (range: 0.5-82 years); 57 patients (32.4%) were children (13 years or younger), and 14 were under three years old. Most of the study participants (164, 94.8%) were of Italian nationality. One hundred and thirty seven patients were recruited in urban areas and 39 in rural villages located in the province of Rome.

Of the 173 samples tested, 74 were positive for one virus and two were positive for two viruses, totalling in 78 viruses detected. The numbers of samples positive for influenza and/or other viruses, and negative samples, is shown in the figure. The most commonly detected agent was influenza virus, which was found in 58 samples (74.4% of all isolates), followed by adenoviruses (11.5%), PIV (7.7%), coronaviruses (3.8%), and RSV (2.6%). Of the influenza isolates, 56 were influenza A (23 of these were typed: 22 were H3N2 and one H1N1), and only two were influenza B strains. With regard to PIV isolates, three were PIV type 3, two were type 4, and one was type 1. Of the samples positive for two viruses, one was positive for influenza and coronavirus, the other for RSV and adenovirus.

Of the 173 samples, 66 were collected during the low-medium influenza activity period (that is, from weeks 46 to 53 and weeks 10 to 17), and 107 during high influenza activity (between weeks 1 and 9). As shown in Table 1, the distribution of the different viral agents differed between the two periods (P = 0.01), due to increased influenza activity in early 2005. The proportion of negative samples was higher in the 'low' activity compared with the 'high' activity period: negative samples were 43 (65.1%) and 54 (48.6%), respectively (P = 0.01).

As shown in Table 2, the proportion of samples positive for influenza viruses was higher in the youngest age group and tended to decrease with increasing age (chi square for trend, P<0.01). Children (≤13 years of age) were more than twice as likely than adolescents over 13 years and adults to be infected with influenza viruses (OR: 2.2, 95% CI: 1.08-4.50). None of the 13 patients aged 65 years or over was positive for influenza viruses.

Overall, 40 of the 173 participants (23.1%) had been vaccinated for influenza: 12 of them (30%) were infected by influenza viruses versus 46 of 133 (34.6%) of non-vaccinated participants; the difference was not statistically significant (OR: 0.81, 95% CI: 0.35-1.85). Among participants younger than 65 years old, 12 of the 30 vaccinated (40%) and 46 of 130 unvaccinated (35.4%) were found to be infected with influenza viruses (OR: 0.82, 95% CI: 0.34-2.00), while none of the 10 vaccinated and the 3 unvaccinated participants aged 65 years or older was positive.

The distribution of symptoms among ILI patients with laboratory confirmed influenza and among the other cases is shown in Table 3: muscle pain (P=0.028) and productive cough (P=0.046) were more likely, and nausea (P=0.045) less likely to be reported in cases positive for influenza viruses; however, no statistical significance remained after applying the Bonferroni correction.

Discussion

In our study, about 44% of the samples were positive for at least one virus. This is fairly consistent with the results of other studies where viruses were detected in a range between 36%-38% (6, 15) and 58% (5). In another study of community-acquired respiratory infections, including also *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in addiction to viral agents (4), at least one potentially pathogenic microorganism was detected in 52% of the swabs.

During the study period, a major influenza epidemic occurred. Thus, in accordance with other studies (4, 6, 15), influenza was the most commonly detected virus. The lack of a protective effect from influenza vaccination was probably due to viral drift leading to the mismatch between wild and vaccine strains [16]. RSV, which was reported to be almost as common as influenza viruses in one of the abovementioned studies, with the highest impact in the youngest age

Table 1

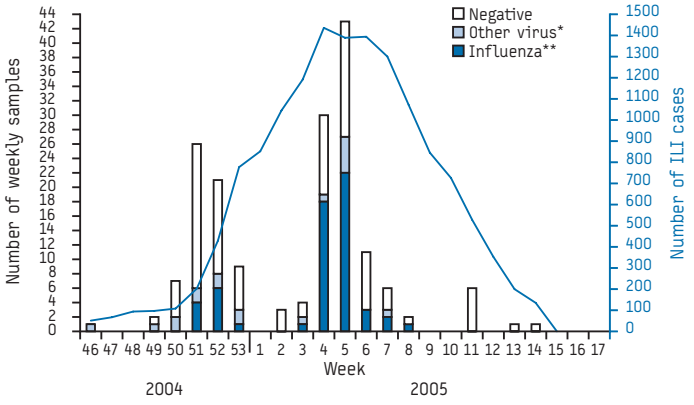
Frequency distribution of specific viral agents by period of sample collection, Rome, 2004-2005

	Weeks 46-53 (2004) and 10-17 (2005)		Weeks 1 to 9 (2005)		Total	
Virus	No.	%	No.	%	No.	%
Influenza	11	47.8	47	85.5	58	74.4
Adenovirus	5	21.7	4	7.3	9	11.5
PIV	4	17.4	2	3.6	6	7.7
Coronavirus	2	8.7	1	1.8	3	3.8
RSV	1	4.4	1	1.8	2	2.6
Total	23	100.0	55	100.0	78	100.0

Note: The percentages are calculated from the total number in each column.

Figure

Cumulative number of positive and negative samples for influenza and other viruses, and number of ILI cases in the province of Rome, winter season 2004-2005



Notes:

* Only one sample is considered for the sample positive for two 'other viruses' (weeks 52 and 53).

** The sample positive for both influenza and another virus is included among influenza positive samples.

Source: FLU – ISS (National Surveillance System)

Table 2

Proportion of samples with laboratory confirmed influenza viruses and samples with other pathogen or no pathogen identified by age class, Rome, 2004-2005

	Samples with laboratory confirmed influenza		Samples with other or no pathogen identified		Total	
Age (years)	No.	%	No.	%	No.	%
0 - 2	7	50.0	7	50.0	14	100.0
3 - 13	19	44.2	24	55.8	43	100.0
14 - 64	32	31.1	71	68.9	103	100.0
>65	0	0	13	100.0	13	100.0
Total	58	33.5	115	66.5	173	100.0

Note: The percentages are calculated from the total number in each row

Table 3

Distribution of signs and symptoms of ILI patients with laboratory confirmed influenza versus ILI patients with other pathogen or no pathogen identified, Rome, 2004-2005

Symptoms	Influenza (n=58*) %	Other (n=115) %	Total (n=173) %
Sore throat	67.2	72.0	70.5
Nasal congestion	67.2	60.0	62.4
Muscle pain	63.8	46.1	52.0
Headache	50.0	39.1	42.8
Dry cough	50.0	53.9	52.6
Productive cough	44.8	29.6	34.7
Chills	37.9	38.3	38.2
Joint pain	29.3	33.9	32.4
Retrosternal pain	29.3	22.6	24.9
Sweating	20.7	25.2	23.7
Short breath	15.5	18.3	17.3
Abdominal pain	8.6	10.4	9.8
Diarrhoea	3.4	6.1	5.2
Nausea	3.4	13.0	9.8
Vomiting	3.4	9.6	7.5

*One patient was positive for both influenza and coronavirus

groups [6], was rarely detected in our survey: this might be due to the low proportion of children recruited, which was itself a result of the low number of paediatricians involved in the survey. Other viral agents, such as adenoviruses, PIV, and coronaviruses were detected in sporadic cases in our study population.

The virological pattern tended to be consistent with the trend of ILI cases reported to FLU-NET in the province of Rome: as expected, influenza viruses were more likely to be detected in the 'high' influenza activity period, whereas the other viruses were only sporadically detected both in the 'high' and in the 'low/medium' influenza activity period. The distribution of the different viral agents varied across age groups, with influenza viruses being more likely to be detected in younger patients.

Before drawing conclusions limits and biases of this study should be mentioned. Firstly, recruitment bias could have affected the results of our study in several ways: i) the consultation pattern of the doctors included in our study was not completely consistent with that of the national surveillance system (FLU-ISS) in the area of Rome; ii) the proportion of children enrolled in our study was relatively low, due to limited participation of paediatricians; iii) irregular sampling, including the lack of recruitments during the Christmas holidays, may have biased the overall distribution of specific viruses during the study period. Thus, to what extent our study population was representative of ILI cases occurred in Rome in the winter 2004/05 remains undefined. Secondly, some viruses, such as rhinoviruses and metapneumoviruses, and bacteria, such as *M. pneumoniae* or *C. pneumoniae*, were not studied. In particular, the inclusion of rhinoviruses might greatly increase virus detection frequency, as indicated by studies reporting a higher proportion of these viruses compared to influenza virus [1], and explain the relatively high proportion of unidentified aetiologies in our study. Nevertheless, our findings do not differ significantly from those of other studies conducted up to now. Thirdly, the potential occurrence of false negative results due to the variable sensitivity of the laboratory techniques, and to the type of biological samples, should not be completely ruled out. Furthermore, timing of collection may have decreased the rate of detection, since some swabs were taken up to 4 days after the onset of symptoms (when viruses may have been cleared, at least in part, by the immune response). The maximum sample delay was set at four days because most patients are not visited before the third day after onset. Finally, the extent to which the case-definition we used was unspecific compared with that provided by the Italian Ministry of Health remains undefined. In particular, we cannot exclude the possibility that the inclusion of patients with milder symptoms may have 'diluted' the frequency of detection of influenza viruses.

In conclusion, we were able to identify the aetiology of about half of the ILI that were reported during the 2004-2005 winter season. Influenza was the most commonly identified agent, while cases attributable to other viruses were sporadic. Although surveillance of respiratory viruses associated with ILI is not sustainable, due to high costs and lack of preventive tools, limited aetiological surveys may provide useful information on the effect of specific agents affecting human populations in the winter season.

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