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Nationwide Surveillance of 18 Respiratory Viruses in Patients With Influenza-Like Illnesses: A Pilot Feasibility Study in the French Sentinel Network

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The aim of the present study was to test the feasibility of integrating the diagnosis of 18 respiratory viruses into clinical surveillance of influenza-like illness using a PCR-DNA microarray detection assay. The study took place in the French Sentinel Network, a nationwide surveillance network of General Practitioners (GPs) representative of French GPs in terms age, location, and type of practice (urban/rural). Three virological laboratories also participated in the study. The study was planned for 5 weeks from January 25, 2010 to February 27, 2010. A subset of 150 Sentinel GPs, located in mainland France, was enrolled to collect clinical data and nasopharyngeal samples from every first patient of the week having a medical visit for influenza-like illness defined as a sudden fever of 39°C or more with respiratory symptoms and myalgia. Sixty-three GPs (42%) collected 103 samples while 87 GPs (58%) did not. GPs did not differ with respect to their age, gender, urban/rural distribution, or years of inscription in the Sentinel Network. Patients included were of a similar age and had similar vaccination characteristics, but were more frequently men than influenza-like illness patients reported to the network during the study period. Sixty-one viruses were detected from 56 of 96 (58%) interpretable samples. The respiratory viruses detected most frequently were metapneumovirus and respiratory syncytial virus. This study showed that virological diagnosis of 18 respiratory viruses can be combined with surveillance of clinical influenza-like illness in general practice. Although feasibility has not been demonstrated yet, it will be evaluated over the winter of 2010–2011. ***J. Med. Virol. 83:1451–1457, 2011.*** © 2011 Wiley-Liss, Inc.

KEY WORDS: respiratory viruses; influenza-like illness; surveillance system; general practitioners

INTRODUCTION

The spread of the new variant A/(H1N1) 2009 influenza virus in the Northern hemisphere at an earlier time than usual for seasonal influenza in previous years has highlighted the limited knowledge concerning the aetiology of acute respiratory illnesses outside of influenza outbreaks.

In early September 2009, while reports of influenza-like illness were increasing in medical practice-based

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surveillance in France and other European countries, the detection of pandemic influenza virus remained sporadic [Linde et al., 2009]. This finding was attributed to the circulation of other respiratory viruses and, to a lesser extent, may have been associated with the increased propensity of patients with influenza-like illness to seek medical advice due to increased anxiety in the pandemic context.

This finding was also observed for influenza-like illness incidence rates reported by the French Sentinel Network [Turbelin et al., 2009]. The Sentinel Network is a continuous epidemiological surveillance system based on General Practitioners (GPs) and has been operating since 1984 in France. It consists of a sample of 1,319 GPs throughout France, of which 200–400 participate each week in continuous surveillance [Valleron et al., 1986]. The GPs participating in the Sentinel Network are representative of the whole French GP population as regards to age, location, type (rural/urban), and kind of practice (alone/pluridisciplinary) [Chauvin and Valleron, 1995].

The main objective of the French Sentinel Network is to provide the national health authorities with near to real-time information on several health-related events occurring in the community, including influenza-like illness (for the full list of events, see http://www.sentiweb.fr). These events are reported by Sentinel GPs through a dedicated website, aggregated at different spatial and temporal levels and used to inform health authorities. In the Sentinel network, influenza-like illness cases are defined on the basis of clinical signs, but no virological collection is carried out routinely.

Given the need for improving virological surveillance after the first A/(H1N1)2009 pandemic wave, which spanned from September 6, 2009 to December 26, 2009 in France, the feasibility of integrating the diagnosis of respiratory viruses into clinical surveillance of influenza-like illness in the French Sentinel Network was tested. As a secondary objective, virological and clinical data were analysed.

METHODS

Study Design and Inclusion Criteria

Three virological laboratories participated in the study. A subset of 150 volunteer GPs located in mainland France was recruited, on the basis of their participation in continuous surveillance in the Sentinel Network.

The 150 GPs were distributed over all the departments (administrative units) of France. The GPs participating to the present study did not differ from other GPs of the Sentinel Network with respect to age (51.7 ± 8.1 years vs. 52.1 ± 7.8 years; $P = 0.93$), gender (male) (79% vs. 83%; $P = 0.60$) urban/rural distribution (urban 75% vs. 74%; rural 25% vs. 26%; $P = 0.68$) and years of inscription in the Sentinel Network (10.4 ± 7.6 years vs. 11.1 ± 7.7 years; $P = 0.27$).

The pilot study was carried out over 5 weeks between January 25, 2010 and February 27, 2010, after the end of the French A/(H1N1)2009 epidemic. The 150 enrolled volunteer GPs received swabs and other study materials to include every first patient of the week having a medical visit for influenza-like illness (Sentinel network case-definition: A sudden fever of 39°C or above, respiratory symptoms and myalgia), with the additional criteria of the time between symptom onset and the visit of less than 48 hr.

Sample and Patient Data Collection

Sample collection was performed with ∑-Virocult® swabs (ELItech, France). The nasopharyngeal swabs were sent by mail in 2 ml of viral transport medium within 2 days to one of the three virological laboratories participating in the study. Detailed demographic and clinical data (time of the onset of symptoms, reported symptoms, physical findings, and influenza vaccination status) were obtained from patients during the medical visit.

Nucleic Acid Extraction and Microarray Detection

Following RNA and DNA extraction from clinical specimens using either an automated MagNA Pure LC with the Total Nucleic Acid High Performance kit (Roche Diagnostic, Meylan, France) or an EZ1 Biorobot with the virus mini kit (Qiagen, Courtaboeuf, France), all samples were tested for the simultaneous detection of a panel of 18 respiratory viral pathogens using a PCR-DNA microarray detection assay (CLART® PneumoVir and FluaVir Version 3.0, Genomica, Madrid, Spain). The CLART® PneumoVir allows the detection of 17 respiratory viruses distinguishing between the seasonal influenza A subtypes A/H1N1 and A/H3N2. The CLART® FluaVir kit was able to type and to distinguish the influenza virus A/H1N12009 from the A/H1N1 and A/H3N2 subtypes of the seasonal influenza A virus. All respiratory samples were tested simultaneously using these two kits.

The combination of these two systems allows for the simultaneous detection of Adenovirus (AV), Bocavirus (hBov), Coronavirus 229E (hCoV), Enterovirus (Echovirus) (EV), Influenza A ((H1N1) 2009, H3N2, H1N1), Influenza B, Influenza C, MetaPneumoVirus (hMPVa and hMPVb), Parainfluenza (PIV) (1, 2, 3, and 4), Respiratory Syncytial Virus (RSVa and RSVb), and Rhinovirus (RV). The technique includes an internal control that tests the efficiency of the amplification process and to detect the presence of inhibition products. The sensitivity and specificity of the multiplex RT-PCR assay for the simultaneous detection of this panel of respiratory viral pathogens have been previously reported. [Renois et al., 2010].

Surveillance virological results (number of samples, positive samples, type and subtype of viruses isolated weekly) were available on the website the week

following sample collection. Each week, the GPs also received the virological results of her/his patients on a secure study website. The interval between specimen collection and availability of the results for GPs was 10–14 days. Finally, an e-mail survey in GPs who did not include any patients to describe the reasons for the lack of sample collection was performed, and another survey was performed to evaluate the GPs' satisfaction with the pilot study in those who included patients.

Statistical Analysis

Patients with at least one virus detected (positive patients) were compared to patients with no virus detected (negative patients). Factors and signs associated with positivity for the viruses that were most frequently isolated or with co-infections were studied. The Fisher exact test was used to compare independent categorical variables and the Mann–Whitney test was used to compare continuous variables. A logistic regression model was used for binomial outcomes, and odds ratios (OR) and their 95% confidence intervals were estimated.

RESULTS

Number of Samples Collected by GPs

Sixty-three (42%) GPs collected 103 nasopharyngeal samples. Based on clinical data reported by the GPs, all samples were collected in patients with influenza-like illness as defined by the surveillance network. The maximum number of samples collected was during week 04 (from January 25, 2010 to January 29, 2010) (n = 36). Thirty-nine (62%) GPs provided one sample, eleven (17%) provided two samples, ten (16%) GPs provided three samples, and three (11%) GPs provided four samples.

Comparison of GPs Who Collected Samples and GPs Who Did Not

GPs who collected samples (n = 63) did not differ from those who did not (n = 87) with respect to their age (51.8 ± 7.4 years vs. 51.7 ± 8.8 years; $P = 0.93$), gender (male) (78% vs. 80%; $P = 0.68$), and years of inscription in the Sentinel Network (10.8 ± 7.2 years vs. 10.7 ± 7.4 years; $P = 0.68$).

GP Satisfaction Survey

Sixty-three percent (40/63) of GPs responded to the satisfaction survey. Thirty-five out of 40 (87.5%) GPs were satisfied with the swab collection instructions and 38 (95%) found the dedicated study website useful for following the development of the study and for consulting instructions. Thirty-four (82.5%) GPs were satisfied with the feedback on the results.

Survey in GPs Who Did Not Collect Samples

Eighty-seven GPs did not collect nasopharyngeal samples. Four items have been proposed to explain the reasons for not participating (lack of any influenza-like illness cases during the study period; lack of time; the study was too complex; declined to participate). Ninety-five percent (83/87) of GPs declared that they did not collect any samples because of the lack of any influenza-like illness case during the study period, but they reported themselves to be very interested in participating in the study. Four GPs did not participate because of a lack of time and they declined to participate in the following phase of the study.

Characteristics of Patients

The median age of the 103 patients with influenza-like illness patients was 18 years [3–75], of whom 58 (56%) were males. These patients did not differ from cases of influenza-like illness reported by the Sentinel network during the study period (n = 380) as regards age ($P = 0.49$) or influenza vaccination status ($P = 0.46$). However, the proportion of men was higher in this sample (56%) than in cases of influenza-like illness reported in the network (45% men) ($P = 0.02$).

Of note, 50% of the included patients were children under the age of 18 which, compared to the proportion of children in the general French population (22%), indicated that children were 3.5 times more likely than adults to be included in the sample of patients with an influenza-like illness.

Virological Findings

Seven samples were excluded since the virological results were not interpretable by the FluaVir (n = 4) microarray or the PneumoVir microarray (n = 3) because of undetectable internal controls. Among the remaining 96 influenza-like illness cases, 61 viruses were detected in nasopharyngeal samples from 56 (58%) patients: RSV in 24 (25%), hMPV in 13 (13.5%), hCoV 229E in 9 (9.4%), Influenza A/H1N1 2009 in 5 (5.2%), RV in 3 (3.1%), hBoV and AV in 2 (2.1%), and Influenza B, Influenza A/H3N2 and PIV 2 in 1 (1.0%) (Table I). More than one virus was detected in four patients (Table I).

The age distribution (0–5; 6–18; 19–25; 26–44; 45–87 years) differed significantly between positive and negative patients, with positive patients being younger than negative patients (OR = 0.98, IC 0.95–0.99; $P = 0.0226$). All patients aged 0–5 years (n = 12) were positive for one or more respiratory viruses, among whom eight had a positive diagnosis for RSVb infection (Fig. 1).

The most common clinical findings in patients infected with hMPV (n = 13) and RSV (n = 24) were cough (n = 11 and n = 24), rhinorrea (n = 11 and n = 22), fatigue (n = 11 and n = 21), and myalgia (n = 10 and n = 16). Co-infected patients (Table I) had a median age of 15 years and did not differ clinically from mono-infected patients.

TABLE I. Viral Aetiology of the ILI Patients

Viral aetiology (co-infections included)	Number	% of patients
Adenovirus	2	2.1
Bocavirus	2	2.1
Enterovirus	0	0
Human coronavirus type 229E	9	9.4
Influenza virus type A subtype H1N1	0	0
Influenza virus type A subtype H1N1 2009	5	5.2
Influenza virus type A subtype H3N2	1	1
Influenza virus type B	1	1
Influenza virus type C	0	0
Metapneumovirus type A	11	13.5
Metapneumovirus type B	2	
Parainfluenza virus type 1	0	1
Parainfluenza virus type 2	1	
Parainfluenza virus type 3	0	
Parainfluenza virus type 4	0	
Respiratory syncytial virus type A	10	25
Respiratory syncytial virus type B	14	
Rhinovirus	3	3.1
Total number of detected virus	**61**	
Details of detected co-infections		
Metapneumovirus A + respiratory syncytial virus A + respiratory syncytial virus B	1	1
Influenza B + metapneumovirus A	1	1
Adenovirus + human coronavirus 229E	1	1
Bocavirus + respiratory syncytial virus A	1	1
Number of positive patients	**56**	**58.3**
Total number of patients	**96**	

DISCUSSION

Despite the small number of samples included, the present pilot study showed that it was feasible to combine microarray detection of 18 respiratory virus infections with clinical surveillance of influenza-like illness in order to provide a more complete understanding of the epidemiology of these viruses in the community.

Combined community virological and clinical surveillance for influenza or RSV have been implemented in several countries. Notably, in France, the Groupes Régionaux d'Observation de la Grippe (GROGs) is an influenza-devoted surveillance system which has been

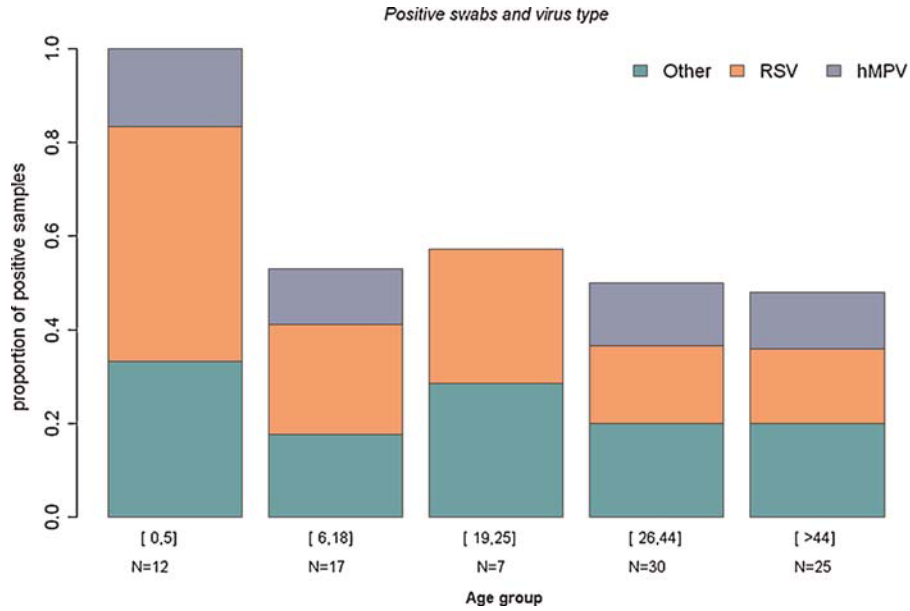

Fig. 1. Aetiology of viral respiratory agents (RSV, hMPV, and the other viruses detected) by age group.

operating for more than 25 years in which GPs or pediatricians perform virological diagnosis of influenza in patients with acute respiratory illness [Quénel and Dab, 1998]. Similar networks have been launched in other European countries, e.g., the UK, Switzerland, Italy, and Spain (www.ecdc.europe.eu). But, to our knowledge, no surveillance system combines the simultaneous diagnosis of a large number of respiratory viruses with clinical surveillance of influenza-like illness using a well-defined case definition.

The simultaneous diagnosis of a large number of respiratory viruses associated with clinical surveillance allows for extrapolation of the prevalence rates of each virus in patients matching the clinical case definition to a nationwide level and facilitates the interpretation of surveillance data. In addition, from a technical viewpoint, the microarray assay requires fewer human interventions (up to 5 hr of hands-on time can be saved compared to qRT-PCR) and allows for the testing of up to 24 samples per run (66 min of setup, excluding RNA extraction and RT-PCR) with the results available on the next day [Frobert et al., 2011; Raymond et al., 2009]. Thus, the surveillance system could be sustained during periods when the number of cases of influenza-like illness is much higher. Although obtaining the results on the same day would be the ideal scenario, the ease of implementation and higher throughput are important factors, especially in high-volume laboratories [Raymond et al., 2009]. The present pilot study was not optimized to shorten the delay between collection and delivering the results to the GPs, the reason why the actual delay was 10–14 days. However, the results of the surveillance were made available the week following sample collection, notably because results were aggregated over the preceding week.

The feasibility of the programme has been questioned, given the fact that only 63 of 150 GPs collected at least one sample. Although this has not been demonstrated yet, it will be evaluated over the winter of 2010–2011. The small number of participants in this first season might easily be explained by the case definition for the collection of specimens, which were rather stringent and included elevated fever. Of note, 146 (97%) GPs have agreed to continue participating in the programme during the winter of 2010–2011.

Among the 103 samples tested, the results of seven samples were not available because of PCR-inhibited or uncertain results. In many cases, the failure in identification could have resulted from a failure in the nucleic acid amplification step rather than from a failure of the microarray [Townsend et al., 2006], even if technical training is necessary to avoid false positive results because of insufficient washing or overexposure of the arrays [Frobert et al., 2011]. Excluding these samples, 58% were positive for at least one virus. This proportion of positive diagnosis is comparable to findings from other studies using qRT-PCR or a combination of qRT-PCR and classical cell culture techniques, in which 41–61.8% of the samples were positive [Follin et al., 2009; Heikkinen et al., 2008; Laguna-Torres et al., 2009; Nougairède et al., 2010].

It is noteworthy that the clinical criteria used for sample collection in those studies included a broad spectrum of respiratory symptoms, while the criteria for influenza-like illness were rather narrow/specific. Therefore, the number of positive specimens was not altered by the selection of patients with a strict case definition. On the other hand, false-negative results may have occurred in the adult population and could have been the consequence of insufficient quality of the sample collected or of a lack of sensitivity of the laboratory techniques. Nevertheless, a recent study validated the performance of the commercial kits used in the present study [Renois et al., 2010].

Negative results can also be due to other unknown or known respiratory pathogens (e.g., Parechovirus, Polyomavirus KI and WU, Human coronavirus OC43, NL63 and KU1, or *M. pneumoniae* and *C. pneumoniae*). This proportion of undiagnosed cases is in line with those found in other surveys [Hasman et al., 2009; Laguna-Torres et al., 2009] and appeal for complementary virological investigations using, for example, high throughput methods (e.g., pyrosequencing methods [Deyde et al., 2009], mass spectrometry [Chen et al., 2011], high resolution melting (HRM) techniques [Varillas et al., 2011], and Padlock Probes [Wu and Tang, 2009]).

In this investigation, it was found that age was associated with the chance of being positive, with a rate of positivity of 100% in younger patients (0–5 years), in which RSV and hMPV were the most frequently detected viruses. Similar findings were obtained in other settings and were attributed to the developing immune status of young children and their vulnerability to infections [Raboni et al., 2011].

Given the temperature criteria used for the influenza-like illness case-definition, children (50%) were overrepresented in comparison with adults relative to the general population (22%). This figure is well in line with current knowledge that fever is usually higher in children than in adults [Feigin et al., 2009].

However, the age distribution of patients did not differ from the age distribution of reported influenza-like illness cases in the network during the period. Therefore, a selection bias related to age was excluded. Moreover, the Sentinel Network involves GPs and does not involve pediatricians, which explains the low proportion of very young children (<2 years) in the sample.

A substantial proportion of adult subjects with RSV (11.5%) or hMPV (8.3%) were found in this study. These findings were also consistent with other reports in which RSV was detected in 11–22% of adult patients during medical visits [Falsey et al., 2006; Zambon et al., 2001] and hMPV in 2.2–10.6% in adult participants during a prospective follow-up [Walsh et al., 2008].

In the present study, there were no significant differences in the clinical characteristics of patients

infected with hMPV, RSV, or other viral infections, but the number of patients in each group was low and comparisons may have been underpowered.

As in previous studies [Heikkinen et al., 2008], the fact that co-infections were common was confirmed. In this study, co-infections with hMPV occurred twice, with RSVa and Influenza B respectively, but they were not associated with more severe signs than mono-infections, as reported elsewhere [Frobert et al., 2011; Wolf et al., 2006], but here again the number of co-infected events was too low to reach a conclusion. In addition, because these RT-PCR DNA microarray detection techniques are only qualitative, not quantitative, it was not possible to determine which virus was predominant in concomitant respiratory viral infections.

In conclusion, although feasibility was not formally demonstrated (e.g., with respect to a set of pre-specified criteria), this study showed that virological diagnosis of 18 respiratory viruses can be combined with surveillance of clinical influenza-like illness in general practice. Such an approach, if used routinely, could help to describe better the seasonal and community burden of these respiratory viruses and to assess the clinical outcome of single and multiple viral infections.

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