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Epidemiological and Virological Assessment of Influenza Activity in Apulia, Italy, During the Seasons 2004 – 2005 and 2005 – 2006

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This study evaluated the impact of influenza and vaccination coverage in Apulia, Italy, during the 2004 – 2005 and 2005 – 2006 seasons, using epidemiological and virological surveillance data collected through the Italian Net of Surveillance of Influenza (InfluNet) organized by the Superior Institute of Health (ISS) and the Inter-University Centre of Research on Influenza (CIRI). Vaccination coverage was calculated from the number of doses administered to individuals aged ≥ 65 years. Sentinel physicians reported weekly influenza-like illness (ILI) and acute respiratory illness (ARI) occurrences among patients. Influenza viruses were isolated and identified by cell culture on Madin-Darby Canine Kidney cells and polymerase chain reaction techniques. Vaccination coverage reached 72.7% and 77.0% during the 2004 – 2005 and 2005 – 2006 seasons, respectively. Incidence of ILI was higher during the 2004 – 2005 season compared with the 2005 – 2006 season, whereas the incidence ARI appeared to show a more constant trend. Incidence rates for ILI and ARI were higher in the 0 – 14-year age group. The increase in vaccination coverage and implementation of a network of epidemiological and virological surveillance are fundamental for the control and prevention of influenza.

KEY WORDS: Influenza; Vaccination; Virology; Epidemiology; Surveillance; Polymerase chain reaction (PCR)

Introduction

Human influenza is a highly contagious acute respiratory tract disease related to severe morbidity and mortality, particularly in elderly or immunocompromised patients.[1] There are three types of human influenza viruses: types A, B and C.[2] Infection with influenza A virus is the most severe, with

See Appendix for details of the Influenza Collaborative Group.

several notable pandemics during the past century.[3] Influenza A viruses are classified into subtypes according to the antigenic composition of their haemagglutinin and neuraminidase glycoproteins on the viral envelope.

The symptoms and signs of influenza infection may be similar to other types of respiratory viral, chlamydiae or *Mycoplasma pneumoniae* infections. Prompt diagnosis of influenza infection would, therefore, facilitate effective patient management, public health and vaccination programmes, as well as the appropriate use of antiviral therapy.[4] The epidemiology of influenza is such that it requires proper surveillance, via exhaustive and complete data collection, on the spread of the disease within the population of a specific geographical area. For this reason, the objectives of this study were: (i) to evaluate vaccination coverage in subjects aged ≥ 65 years; (ii) to evaluate the incidences of influenza-like illness (ILI) and acute respiratory infections (ARI); and (iii) to identify the types and subtypes of the influenza virus circulating in Apulia, Italy, during the 2004 – 2005 and 2005 – 2006 seasons.

Subjects and methods

VACCINATION COVERAGE

In Italy, the target for vaccination coverage set by the National Health Plan (PNS) 1998 – 2000[5] and reiterated in the National Vaccines Plan 2005[6] is 75% in the population aged ≥ 65 years. Influenza vaccination is offered to people aged ≥ 65 years and to all individuals belonging to at-risk categories (children aged 0 – 4 years, adults with chronic cardiovascular or respiratory diseases, patients with diabetes mellitus, etc.) as specified in the annual circular issued by the Italian Ministry of Health.[7,8]

The numbers of individuals in each of the AUSL (Azienda Unità Sanitaria Locale) of Apulia region, Italy, who were aged ≥ 65 years and vaccinated for influenza were collected for the influenza seasons 2004 – 2005 and 2005 – 2006. The rates of vaccination coverage for each season and AUSL were then estimated as follows:

$$\text{Vaccination coverage (\%)} = \frac{\text{Vaccinated individuals aged} \geq 65 \text{ years}}{\text{Total population aged} \geq 65 \text{ years}} \times 100$$

EPIDEMIOLOGICAL SURVEILLANCE

During the influenza seasons 2004 – 2005 and 2005 – 2006, the Italian Surveillance Influenza Network (InfluNet) was in operation, organized by the Superior Institute of Health (Istituto Superiore di Sanità; ISS) and the Inter-University Centre of Research on Influenza (CIRI). This surveillance system involved a group of sentinel doctors (paediatricians and practitioners) who were required to survey at least 1% of the total population of Italy.

In Apulia, the system of surveillance started on week 42 of each year and continued until week 17 of the following year. Data on the number of cases of ILI and ARI among patients, and whether or not those patients had or had not been vaccinated against influenza, were collected by the sentinel doctors and sent weekly to the CIRI via electronic data transmission. The number of ILI cases was based on a standard case definition: abrupt onset of fever (≥ 38 °C); one or more respiratory symptom (non-productive cough, sore throat, rhinitis) and one or more systemic symptom (myalgia, headache and severe malaise). The standard case definition for ARI was the occurrence of one or more of the following: rhinitis, rhinopharyngitis, tonsillitis, laryngotracheitis, tracheitis or acute bronchitis.

VIROLOGICAL SURVEILLANCE

At the same time as the clinical-epidemiological surveillance, a virological surveillance was also carried out, and involved doctors who were not necessarily enlisted for InfluNet.

In each influenza season (2004 – 2005 and 2005 – 2006), from week 46 until week 17 of the following year nasopharyngeal samples were collected from a relatively limited number of patients fitting the criteria for ILI. Samples were collected during the acute phase of the disease (febrile period) using Virocult® sterile swabs (Medical Wire & Equipment, Corsham, UK). RNA extraction was carried out using the RNeasy Qiagen kit (Qiagen, Hilden, Germany) following the operative protocol of the supplier.

For samples collected in the 2004 – 2005 season, reverse transcription-polymerase chain reaction (RT-PCR) was performed using the Influcheck diagnostic kit (Euroclone, Milan, Italy). This allowed identification, typing and sub-typing of the influenza virus (Orthomyxoviridae) through the gene amplification of the regions that are stored in the matrix protein and in the haemagglutinin. In the 2005 – 2006 season, identification of the influenza virus was performed using real time PCR (Fast Set InfA/InfB, Arrow Diagnostics, Genova, Italy). This enabled identification of the RNA for influenza virus types A and B by RT-PCR *in vitro* in a single tube. Subtyping of samples that were positive for influenza type A through real time PCR was performed using RT-PCR with the Influcheck diagnostic kit.

Components of the influenza virus were successively isolated by cell culture, in collaboration with the Department of Health Sciences, Section of Hygiene and Preventive Medicine, University of Genoa, Genoa, Italy, in a method comprising three blind steps, each of 7 days' duration, on a monolayer of Madin Darby Canine Kidney (MDCK) cells.

Results

VACCINATION COVERAGE

The rates of vaccination coverage for individuals aged ≥ 65 years in each of the AUSL in Apulia during the two influenza seasons (2004 – 2005 and 2005 – 2006) are shown in Table 1. During the 2004 – 2005 season, the vaccination campaign resulted

TABLE 1:
Rates of vaccination coverage for individuals aged ≥ 65 years in each Azienda Unità Sanitaria Locale (AUSL) in Apulia, Italy, during the influenza seasons of 2004 – 2005 and 2005 – 2006

AUSL	2004 – 2005	2005 – 2006
Bari 1	64.8	61.0
Bari 2	77.8	70.9
Bari 3	45.5	78.7
Bari 4	72.7	80.0
Bari 5	64.6	99.8
Brindisi 1	69.8	73.6
Foggia 1	68.2	71.3
Foggia 2	78.7	82.8
Foggia 3	83.2	96.6
Lecce 1	71.7	73.2
Lecce 2	68.4	70.1
Taranto 1	71.1	72.6

in a coverage rate of 72.7%. The national target of 75%[5,6] for subjects aged ≥ 65 years, was reached by three AUSL: Bari 2 (77.8%), Foggia 2 (78.7%) and Foggia 3 (83.2%).

The vaccination campaign for individuals aged ≥ 65 years in Apulia during the 2005 – 2006 season registered a vaccination coverage rate of 77.0%. The target of 75%[5,6] was reached by five AUSL: Bari 3 (78.7%), Bari 4 (80.0%), Bari 5 (99.8%), Foggia 2 (82.8%) and Foggia 3 (96.6%). In the other AUSLs of the region, even if the target fixed by the National Health Plan was not attained, the rates of vaccination coverage were higher than in the previous season with the exception of just two areas, Bari 1 and Bari 2.

EPIDEMIOLOGICAL SURVEILLANCE

During the two influenza seasons, through a network of 157 sentinel doctors, about 5.7% of the population, stratified by age class, was monitored in Apulia.

The weekly incidence of ILI in Apulia during the two seasons is shown in Fig. 1. In the 2004 – 2005 season a low incidence was reported until week 52 of 2004 after which an increase was observed, reaching a peak at week 6 of 2005 (11.01 cases/1000 subjects). The ILI incidence for the 2005 – 2006 season was much lower than that of the previous season, peaking in week 13 of 2006 (2.72 cases/1000 subjects).

During the 2004 – 2005 season, the incidence of ILI was greatest in the 0 – 4-year and 5 – 14-year age groups, with maximum incidence of 24.31 cases/1000 subjects (week 6 of 2005) and 18.92 cases/1000 subjects (week 5 of 2005), respectively (Fig. 2A). During the 2005 – 2006 season individuals in these age groups were also most affected; maximum incidence rates of ILI occurring at week 12 of 2006 in the 0 – 4 years age class (7.25 cases/1000 subjects) and at week 13 of 2006 in the 5 – 14 years age class (8.03 case/1000 subjects) (Fig. 2B).

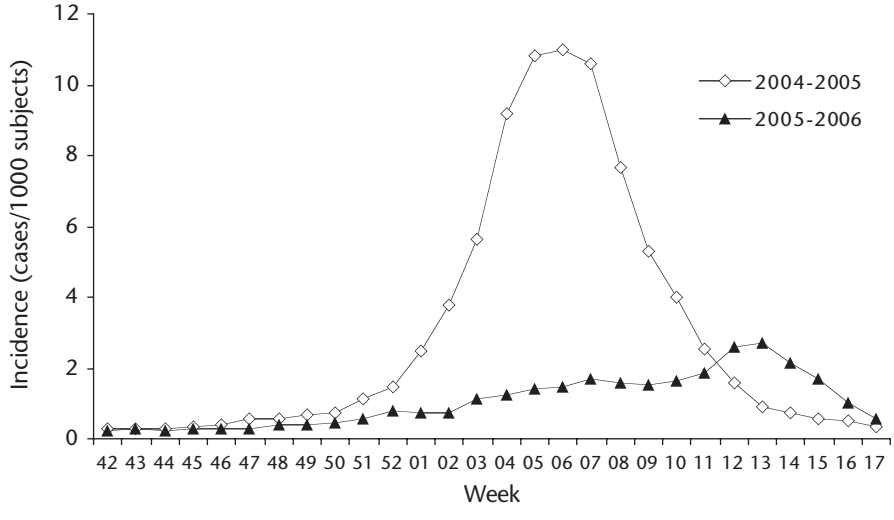

FIGURE 1: Total incidence of influenza-like illness, recorded weekly by sentinel doctors, in Apulia, Italy, during the influenza seasons of 2004 – 2005 and 2005 – 2006

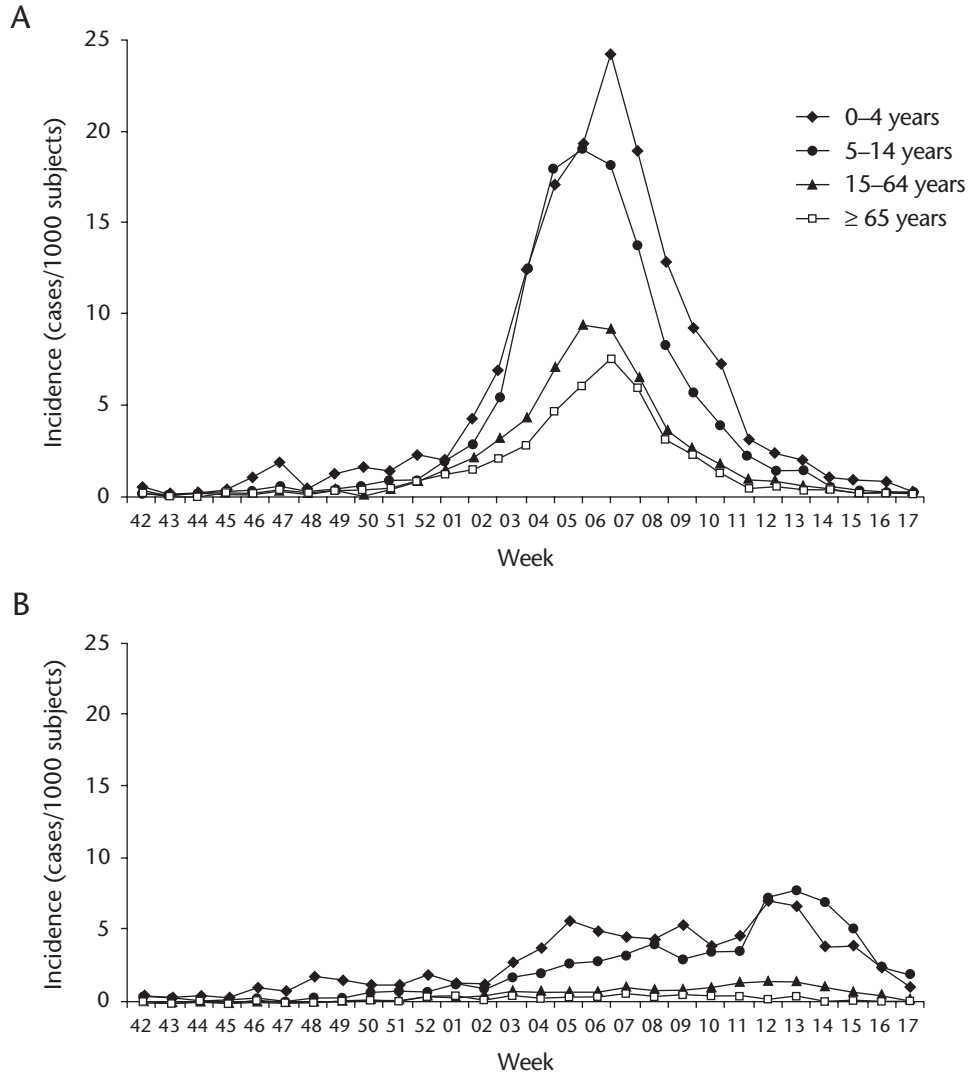

FIGURE 2: Incidence of influenza-like illness, recorded weekly by sentinel doctors, for age groups 0 – 4, 5 – 14, 15 – 64 and ≥ 65 years in Apulia, Italy, during influenza seasons (A) 2004 – 2005 and (B) 2005 – 2006

The incidence of ARI during the influenza season 2004 – 2005 remained fairly constant during the whole surveillance period; on average 3.47 cases/1000 subjects were registered, with higher incidence rates occurring in the 0 – 4-year and 5 – 14-year age groups (peak incidence rates of 14.23 cases/1000 subjects in week 9 of 2005 and 8.71 cases/1000 subjects in week 6 of 2005, respectively) (Fig. 3A). During the 2005 – 2006 season, the ARI rates also showed higher incidence rates in the 0 – 4-year and 5 – 14-year age groups (peak incidence rates of 14.88 cases/1000 subjects in week 6 of

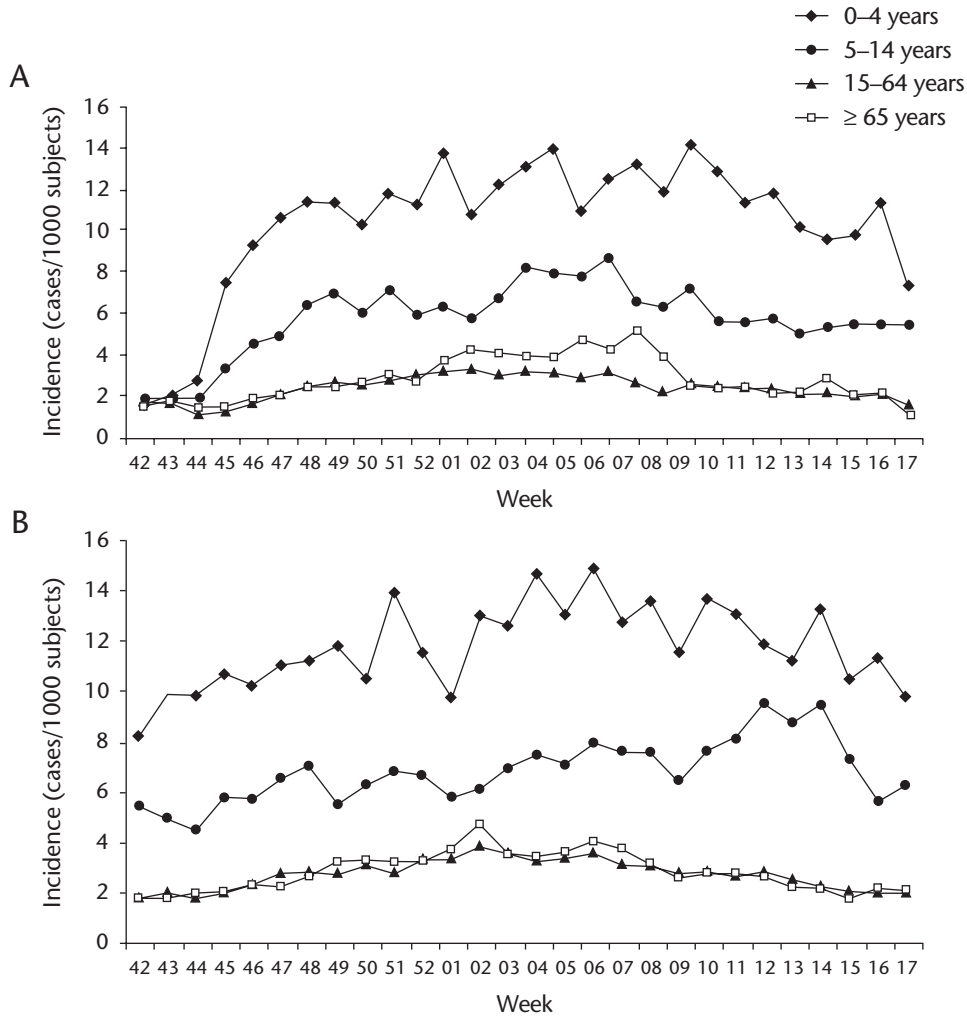

FIGURE 3: Incidence of acute respiratory illness, recorded weekly by sentinel doctors, for age groups 0 – 4, 5 – 14, 15 – 64 and ≥ 65 years in Apulia, Italy, during the influenza seasons (A) 2004 – 2005 and (B) 2005 – 2006

2006 and 9.53 cases/1000 subjects in week 12 of 2006, respectively) (Fig. 3B).

VIROLOGICAL SURVEILLANCE

Virological surveillance was carried out by 11 doctors during the 2004 – 2005 influenza season and by 15 doctors during the 2005 – 2006 season.

During the 2004 – 2005 season, 83 nasopharyngeal samples were collected; 30 were positive in the PCR test, five of which originated from patients who had been vaccinated. Characterization of these positive samples showed the exclusive

presence in all 30 samples of the type A-H3N2 influenza virus.

During the 2005 – 2006 season, 139 nasopharyngeal samples were collected; 18 were positive in the PCR test, five of which originated from patients who had been vaccinated. The characterization of the positive swabs showed a greater variety of influenza virus types: A-H3N2 (n = 8) and B-H3N2 (n = 9) and one only case of type A-H1N1.

Discussion

The wide distribution of influenza viruses, their high capacity to spread and their antigenic instability mean that influenza is an important public health issue. The circulation of new antigenic variants, resulting from antigenic drift, sustains annual epidemics with high morbidity and increases in hospitalization and mortality.[9] Most of the deaths that occur as a result of complications associated with influenza are generally in individuals aged ≥ 65 years and are primarily related to the spread of the virus A-H3N2.[10] In children, infection rates can reach as high as 30%, causing significant increases in the need for medical treatment and hospitalization.[11]

The high rates of spread of influenza viruses along with their short period of incubation make it extremely difficult to develop prompt control strategies and to limit spread of the disease. Vaccination is, therefore, still considered the best method of influenza prevention and control. In Italy, vaccine prophylaxis is carried out annually through specific vaccination campaigns promoted by the Ministry of Health. Vaccination is actively offered and is free to individuals aged ≥ 65 years, and to individuals of any age who have a high risk of complications, such as patients with renal failure, cardiopathy, diabetes mellitus, etc. Vaccination is carried out by the network of vaccination centres or by general medical practitioners.

The circulation of new antigenic variants of the influenza virus necessitates the vaccine composition to be constantly updated. In order to achieve this, the Influenza Worldwide Surveillance Program, coordinated by the World Health Organization (WHO), and in which Italy is participating through the ISS, assesses the actual spread of infection, identifies new antigenic variants, determines their potential and defines the formulation of the vaccine for the next season.[12,13] The prevention of influenza is based on annual vaccination with an inactivated virus vaccine, the effectiveness of which depends largely on the match between vaccine strains and circulating viruses.

During the 2004 – 2005 season in Italy, antigenic characterization testing showed that there was a substantial correspondence between the circulating strains of influenza virus and the vaccine strains A/New Caledonia/20/99 (H1N1) and B/Shanghai/361/2002. Antigenic differences were observed for the subtype A-H3N2, however, which showed antigenic drift that replaced the A/Fujian/411/2002 strain with the new A/California/7/2004 variant.[7]

During the 2005 – 2006 season in Italy, the antigenic and molecular characterization testing of isolates showed a substantial correspondence between the circulating strains and the vaccine strains A/New Caledonia/20/99 (H1N1). Antigenic differences were observed for the subtype A-H3N2 and for the type B viruses. With regard to the A-H3N2 virus circulating during this season, a new variant, A/Wisconsin/67/05, appeared and predominated over the vaccine strain A/California/7/2004. For the type B viruses, the ones with Victoria-like lineage appeared similar to the

B/Malaysia/2506/04 strain, whilst the ones belonging to the lineage Yamagata-like were similar to the vaccine strain B/Shanghai/361/2002.[8]

For the last two influenza seasons of 2004 – 2005 and 2005 – 2006 the national and regional surveillance network of InfluNet has gathered reliable data about the evolution and spread of annual influenza epidemics in Italy. Data from previous influenza seasons have indicated that ILI incidence was highest during the circulation of the type A-H3N2 strains (1999 – 2000, 2002 – 2003, 2003 – 2004 and 2004 – 2005), lowest during the circulation of type B virus (2001 – 2002 and 2005 – 2006), and intermediate during the circulation of the type A-H1N1 (2000 – 2001).[14] Stratification by age for those patients who were positive for influenza virus on laboratory diagnosis showed that the 0 – 4-year and 5 – 14-year age groups had the greatest rates of infection.[7] These data confirm that children, because of their lack of antibodies, represent the main source of infection for the population in general.[14 – 16]

The regional data for Apulia compare well with the Italian national data. In fact, in Italy during the 2004 – 2005 season the incidence of ILI was above the expected mean and the three values of weekly incidence that corresponded to the peak of the epidemic (13.73, 14.59 and 12.72 cases/1000 subjects, registered in weeks 15, 16 and 17, respectively), were the highest of all peak values registered in the last six seasons of surveillance. Virological surveillance allowed the identification of circulating viruses and, during the 2004 – 2005 season, circulating strains in Italy were basically of type A-H3N2, while in the 2005 – 2006 season the circulating strains were of type B.[1] Influenza virus positive samples were collected, almost exclusively, from non-vaccinated subjects.

These results confirm the importance of epidemiological and virological monitoring of influenza and the necessity to ensure prompt identification of epidemic peaks and the characterization of new circulating viral strains.

Appendix

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Conflicts of interest

No conflicts of interest were declared in relation to this article.

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