Surveillance of influenza in a city of North Italy from 1997 to 2002

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Key words

Influenza-like illness • Virological surveillance • Epidemic seasons

Summary

As part of the national program of virological influenza surveillance, influenza activity was monitored in a city of North Italy during five epidemic seasons (1997-2002) via clinical samples obtained from patients presenting with influenza-like illness (ILI).
The aim was to detect early activity and identify influenza strains circulating in each epidemic season.
The virological surveillance activity involved sentinel General Practitioners (GPs) identifying patients with ILI.
During the five seasons considered, annual influenza epidemics occurred in our city, with little differences in time and severity.
Influenza A viruses isolated in this period were predominantly of the influenza A/H3N2 subtype, although in the 2000-2001 season influenza A/H1N1 was most prevalent.
Sporadic cases of influenza A/H1N1 have also been reported during 1999-2000 influenza season, whilst influenza outbreak during the last season considered (2001-2002) was associated predominantly with influenza B.
This study emphasizes the value of virological surveillance for the monitoring and prevention of influenza, providing isolates for further antigenic and genetic characterization and also for vaccine preparation.

Introduction

Influenza is an important problem of Public Health worldwide.
From a public health point of view, influenza is one of the most spreading and burdensome infectious diseases, due to the high morbosity and mortality that this disease can cause in particular groups of population.
The ability of influenza viruses to change rapidly their antigenic equipment allows them to circumvent the immunity acquired as a result of previous natural infections or via vaccination [1-5].
Therefore they are able to cause annual epidemics and infrequently pandemics.
Influenza vaccination is the primary method for preventing influenza and its severe complications [6 7].
Frequent updating of the influenza vaccine content is necessary; only a vaccine whose virus strains match the circulating influenza viruses will protect recipients efficiently from influenza disease.
One of the purpose of a strong virological surveillance of influenza is just identify, as far as possible, new viral strains in circulation.
This surveillance is particularly important if related to the actual epidemiological trend, which puts together the co-circulation, in the same epidemic season, of antigenically different viruses with the global alert for the emergence of influenza viruses with pandemic potential.
It has been almost 36 years since the last influenza pandemic, and the longest interval between pandemics, recorded with certainty, is 39 years.
Recently, the threat of a next influenza pandemic was also highlighted by several human influenza outbreaks caused directly by avian influenza viruses [8-10].
In 1997 a total of 18 Hong Kong residents were infected with A/H5N1 influenza viruses, 6 of whom died and in 2004 there have been other cases of H5N1 influenza in humans residing in Vietnam and China; in 1999, also in Hong Kong, two children suffered mild influenza-like illnesses associated with influenza A/H9N2 virus; in 1999 influenza A/H7N7 virus caused mild conjunctivitis and human influenza A/H1N2 reassortant viruses reappeared during the 2001-2002 epidemic season [11-19].
A regular surveillance of influenza activity plays therefore a key role in the early recognition of new subtypes or new antigenic variants of influenza viruses, in order to predict their ability of spreading and to implement adequate strategies of containment and prevention.
To this end the World Health Organisation (WHO) has created a global clinical-epidemiological and virological influenza surveillance network coordinated in Italy by the Istituto Superiore di Sanità [20-22].
We report virological surveillance data collected during five influenza epidemic seasons (1997-2002) in Parma.

Methods

Collection of specimens

Specimens for diagnosis were collected from November (44th week) to April (16th week) of the following year.

Nasopharyngeal swabs were carried out from patients with clinically diagnosed influenza-like illness (ILI), on reference of the sentinel practice network made up of general practitioners (GPs).
The clinical case definition of ILI used was the same as that recommended by the Ministry of Health, i.e. fever > 38°C, one or more respiratory symptoms, such as cough, sore throat, nasal congestion and one or more general symptoms, such as shivers, malaise, myalgia and a state of prostration [6-23].
Specimens for isolation have generally been taken during the first 3 days after onset of clinical symptoms for influenza and processed within 24 hours.
General practitioners participating to the surveillance of influenza were the same for all five epidemic seasons examined.
Table I shows details of specimens collected during the five-years surveillance period.

Isolation of influenza virus

Madin-Darby canine kidney (MDCK) cells are the preferred cell line for isolating influenza viruses.
Thus, specimens collected were inoculated in culture tubes with MDCK cells; other types of cell cultures (Hep-2 cells, rhesus monkey kidney cells, human embryo fibroblasts) have been used for each specimen, in order to detect different respiratory viruses.
The culture tubes were incubated at 36°C and were examined daily for cytopathic effect (CPE).
For MDCK culture tubes, hemadsorption (HAD) tests and hemagglutination tests were performed on 2-6 days after inoculation, with a guinea pig red blood cell suspension [24-26].

Identification of influenza isolates

Subtype identification of hemagglutinating isolates was carried out by hemagglutination-inhibition (HAI) tests, using polyclonal chicken antisera against influenza A and B reference strains and by reverse transcription-polymerase chain reaction (RT-PCR) amplification, using type/subtype-specific primers [20-22].
RNA from the isolates examined in this study was extracted with either the Trizol LS reagent (Invitrogen, Carlsbad, CA), or the QIAmp Viral RNA mini kit (Qiagen) before reverse transcription-polymerase chain reaction (RT-PCR) amplification. The RT-PCR amplification was performed with either a one-step or a two-step RT-PCR reaction.
The isolated strains were sent to the Istituto Superiore di Sanità in Rome, at the WHO National Collaborating Centre for influenza and from here to the International WHO Collaborating Centre in Mill Hill in London, where the positive specimens have been further characterized using HAI tests with post-infection ferret sera.
Identification of other respiratory viruses, i.e. adenoviruses, enteroviruses, herpesviruses, which have been isolated in clinical specimens during influenza surveillance period, was carried out by neutralization assays in culture cells using rhesus monkey kidney cells or human diploid cells.

Results

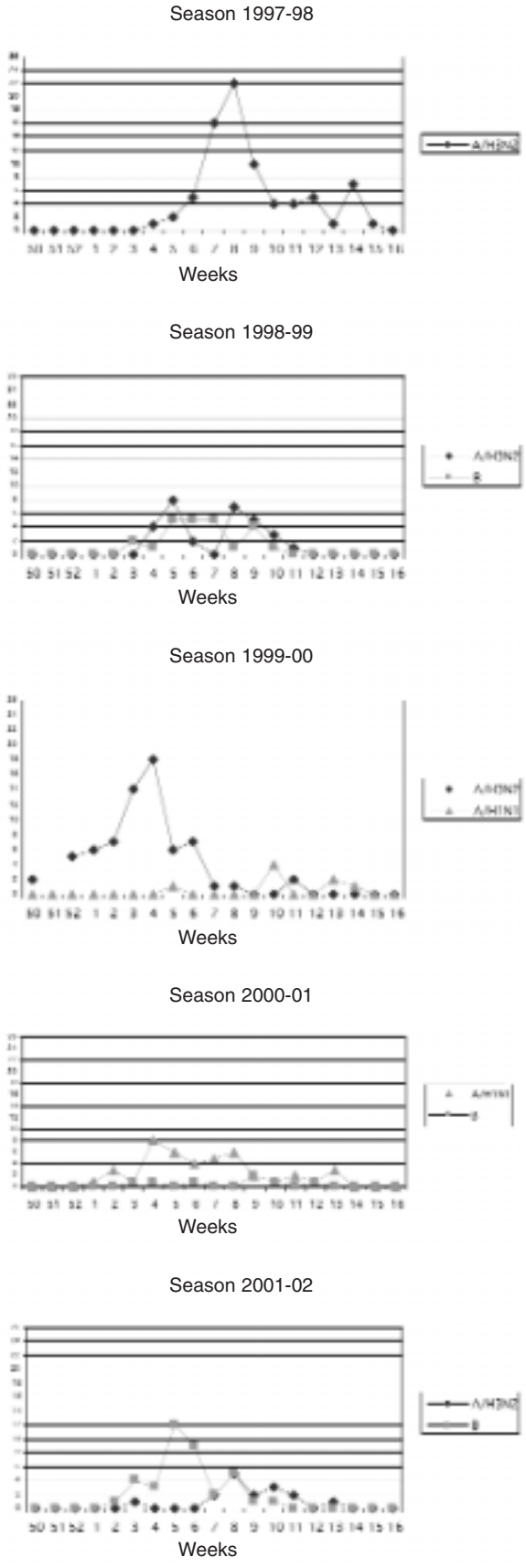
Results of the influenza virological surveillance in Parma, concerned five epidemic seasons (1997-2002), are shown in Table II and Figure 1.
With the exception of the 1999-2000 winter season, in which influenza appeared on the first decade of December, influenza epidemics occurred, in general, from the first week of January to the first week of April (mean length = 13.8 weeks; range = 10-19).
The most important epidemics were recorded during 1997-98 and 1999-2000 seasons, in which isolation of virus increased as a proportion of total respiratory specimens taken during the surveillance period.
There was only little variation in the peak weeks of influenza activity: isolation of influenza viruses peaked in all five epidemic seasons during the month of February (Fig. 1).
In the 1997-98 season, 25.7% of total swabs examined (n. = 305) was positive and 78 influenza viruses were

Tab. I. Sampling.

Influenza season	Sentinel practitioners recruited	Total	Samples Age group				Sex		Vaccinated
	N.	N.	0-4 N.	5-14 N.	15-64 N.	> 64 N.	Male N.	Female N.	N.
1997-1998	4-6	305	18	32	229	26	155	150	ND*
1998-1999	4-6	175	3	11	143	18	93	82	4
1999-2000	4-6	279	61	51	148	19	166	113	9
2000-2001	4-6	189	47	55	82	5	103	86	9
2001-2002	4-6	138	41	45	51	1	78	60	7

* not determined

Fig. 1.



isolated: in all cases the virus identified belonged to subtype A/H3N2.

Antigenic analysis (subtyping) by hemagglutination-inhibition (HAI) tests using selected reference ferret antisera showed that most of 78 isolates were antigenically similar to A/Wuhan/359/95 (current vaccine strain), although some of the influenza viruses isolated reacted best with the serum against A/Sydney/5/97, which was a new variant distinguishable from the reference strain.

During 1998-99 influenza season, 175 swabs were examined, 30.9% (n. = 54) of whom resulted positive.

The thirty influenza A/H3N2 viruses isolated were all closely related to A/Sydney/5/97 reference strain, as well as the twenty four influenza B viruses isolated, which were antigenically close to the current vaccine strain (B/Beijing/184/93).

From November 1999 to April 2000 (the 1999-2000 influenza season), 279 swabs were collected and, in total, influenza A was isolated from 77 individuals (27.6%).

From 77 isolates that were characterized, 69 were antigenically related to an influenza A/Sydney/5/97 (H3N2)-like virus, and eight to an A/Beijing/262/95 (H1N1)-like virus.

The composition of influenza vaccine for the 1999-2000 season included both these strains. In the 2000-01 winter, 50 (26.4%) influenza viruses were isolated from a total of 189 samples collected.

The 43 A/H1N1 viruses isolated were antigenically similar to A/New Caledonia/20/99, which was the currently used vaccine virus.

The seven influenza B viruses were antigenically distinguishable from the B/Beijing/184/93 reference strain and were closely related to the new variant B/Sichuan/379/99.

The 2001-2002 season saw the lower number of samples collected (n. = 138) but the highest percentage of influenza viruses isolated (39.1%).

From 54 isolates, sixteen influenza A/H3N2 viruses were recovered and in the remaining 38 positive samples influenza B virus was found.

In hemagglutination-inhibition (HAI) tests with post-infection ferret sera, the majority of influenza A/H3N2 isolates was similar in titre to the current vaccine strain A/Moscow/10/99 and to other new variants, which were also related or indistinguishable from the vaccine virus.

Analysis concerning the age distribution of patients examined (Tab. I) showed that influenza primarily affected the age groups of youths and adults (15-64 years), in particular during the period 1997-2000, and scarcely affected older age group (65 years and over), especially in the last two influenza seasons (2000-2002).

Tab. II. Virological surveillance during five influenza seasons (1997-2002).

Influenza season	Virus n.			Total isolated virus		Total pharyngeal swabs
	Type A		Type B	N.	%	N.
	Subtype H3N2	Subtype H1N1				
1997-1998	78	0	0	78	25.7	305
1998-1999	30	0	24	54	30.9	175
1999-2000	69	8	0	77	27.6	279
2000-2001	0	43	7	50	26.4	189
2001-2002	16	0	38	54	39.1	138

Discussion and conclusions

During the period considered (1997-2002), annual influenza epidemics occurred in our city, with little differences in time and severity.

Influenza A viruses isolated in this period were predominantly of the influenza A/H3N2 subtype, although in the 2000-2001 season influenza A/H1N1 was most prevalent.

Sporadic cases of influenza A/H1N1 have also been reported during 1999-2000 influenza season, whilst influenza outbreak during the last season considered (2001-2002) was associated predominantly with influenza B.

Altogether influenza activity appeared moderate and data recorded in all five seasons are comparable to those observed in the nation and in the most part of world during the same period [21-23 28-30]. In the same way data concerning the circulating viruses in this five-years period are comparable to national and international ones.

In 1997-1998 winter, countries in the Northern Hemisphere have reported moderate influenza epidemics, associated predominantly with influenza A/H3N2; many of the influenza A/H3N2 viruses isolated were closely related to A/Wuhan/359/95 reference strain although a number of isolates showed antigenic similarity with the new variant A/Sydney/5/97.

Consequently, trivalent vaccines used in the 1998-99 season contained an A/Sydney/5/95(H3N2)-like strain.

Influenza B and A/H1N1 viruses were only isolated from sporadic cases.

Similar results have also been reported throughout our nation.

It is important to mention the 1997 Hong Kong outbreak caused by A/H5N1 virus.

During the 1998-99 influenza season, there has been a co-circulation of influenza A and B viruses. In several countries of western/eastern Europe influenza epidemic was predominantly supported by influenza A/H3N2 subtype, whereas in Italy and in other countries influenza B was most prevalent.

Also in Parma there was a consistent increase in the reporting of influenza B virus infections when compared with the previous season.

Increasing influenza activity, associated with influenza A/H3N2, was reported in the following epidemic season (1999-2000).

Influenza A/H1N1 viruses were associated with outbreaks and in some countries influenza B viruses co-circulated at low levels with influenza A.

The majority of influenza A/H3N2 isolates, in the Northern Hemisphere and also in Italy, was antigenically closely related to the new variant A/Moscow/10/99, although many of these isolates were also closely related to the current vaccine strain A/Sydney/5/97. In the same way, influenza A/H1N1 viruses were antigenically related either to A/New Caledonia/20/99 (new variant) or to A/Beijing/262/95.

For this reason, it was recommended that vaccines to be used in the 2000-2001 season contained an A/New Caledonia/20/99 (H1N1)-like virus and an A/Moscow/10/99 (H3N2)-like virus.

In 2000-2001, influenza epidemic was mainly due to influenza A/H1N1 viruses, which co-circulated with influenza B viruses in some countries, included Italy. Influenza A/H3N2 viruses were isolated sporadically.

In hemagglutination-inhibition (HI) tests with postinfection ferret sera, most influenza A/H1N1 viruses and the few A/H3N2 isolates were antigenically similar to the current vaccine strains, respectively A/New Caledonia/20/99 and A/Moscow/10/99.

The majority of B viruses were antigenically closely related to the new variant B/Sichuan/379/99; thus, influenza B vaccine strain recommended to be used in the 2001-2002 season was changed.

Between November 2001 and April 2002, influenza A viruses predominated in some countries of the Northern Hemisphere, while influenza B viruses predominated in others. In several countries both viruses co-circulated.

In Italy, sporadic cases associated with influenza subtype A/H3N2 were reported, but influenza activity was mainly due to influenza B. Some B viruses isolated, included those isolated in Parma, were antigenically related to the new variant B/Hong Kong/330/2001, which

has become the vaccine strain recommended to be used in the following season (2002-2003).
Data recorded during this five-years influenza surveillance period in Parma, show that influenza A/H3N2 viruses, influenza A/H1N1 viruses and influenza type B have co-circulated among the population, even if all three have not been isolated contemporaneously in the same epidemic season. Each of these three influenza viruses has predominated in different seasons or in a few cases has co-circulated with one of the other.
Surveillance data also report that, in all five epidemic seasons, patients aged 15-64 years have been the most affected with influenza, pointing out the absence, in the period considered, of a link between type/subtype of influenza virus in circulation and age of subjects infected. In the same way, there is no evidence of a link between type of infecting virus and severity of symptoms.
On the contrary, annual influenza epidemic had a relatively small impact on the older age group (65 years and over), especially during the last two epidemic seasons considered. This is maybe due to the good levels of vaccination coverage rate achieved in the over 64 years.
In three (1999/2000, 2000/01, 2001/2002) of the four seasons, in which there was a co-circulation of two influenza viruses, is remarkable a particular temporal appearance of one virus compared to the other. One type/subtype of influenza virus started to spread among the population, causing the beginning of epidemic; the second one followed or replaced the first one later.
Important to be noted is the length of seasonal influenza epidemic. In the five-years period considered, influenza activity lasted an average of 13 weeks, resulting in a substantial seasonal burden to the healthcare system.
Data obtained with this kind of surveillance system (based on notification of sentinel practitioners participating to the surveillance) concern a selected sample of the population, resulting in a small degree of approximation of data.
Nevertheless, we are confident that data recorded are a good representation of influenza transmission, as the general practitioners participating to the surveillance of influenza were the same for all five epidemic seasons examined.
Furthermore, data recorded during this study confirm that influenza virus isolation in MDCK cells is a highly sensitive and very useful technique for the diagnosis of this respiratory infection, providing at the same time isolates for further antigenic and genetic characterization and also for vaccine preparation.

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