

RESEARCH PAPER

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Fifteen years of epidemiologic, virologic and syndromic influenza surveillance: A focus on type B virus and the effects of vaccine mismatch in Liguria region, Italy

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ABSTRACT

In order to estimate the burden of influenza and to describe the genetic evolutionary pattern and antigenic variability of type B viral strains, data deriving from 3 surveillance systems active in Liguria region, Northern Italy, were described. Since the re-emergence of the Victoria lineage in 2001, the clinical-epidemiological and syndromic surveillances demonstrated the heavy burden of influenza like illness (ILI) syndrome. Focusing on type B influenza virus, it predominated or played a relevant epidemic role in the 50% of the evaluated influenza seasons. Furthermore, the virologic surveillance demonstrated the frequent co-circulation of both lineages an heterogeneous circulation of different influenza B strains, determining a partial or complete mismatch in at least 6 influenza seasons. The undemonstrated cross-reactivity between lineages and the unpredictability of predominant lineage arose the scientific debate about the opportunity to include the quadrivalent influenza vaccine among the preventive tools to improve the protection against type B viruses. The integration of different surveillance systems highly contribute to estimate the poorly evaluated burden of type B influenza virus and help to find variants to include in the vaccine formulation.

KEYWORDS

epidemiology; influenza; mismatch; surveillance; vaccine

Introduction

Seasonal influenza epidemics are associated with a heavy health and societal burden worldwide, being responsible for 3–5 million cases of severe illness and about 250–500,000 estimated deaths each year.¹ Moreover, their large economic impact includes not only direct medical costs, but also reduced quality of life and loss of work productivity.^{2–5}

Until recent times, influenza A conventionally remained the primary focus of influenza control, because of its pandemic potential and its predominance in most seasonal influenza epidemics.^{6–8} Nevertheless, during interpandemic periods, influenza B could account for a relevant proportion of cases.^{9–14}

Influenza B viruses evolutionary pattern extremely differs from that of type A viruses. In particular, humans are the only host of epidemiological relevance for influenza B viruses. The main consequence of this fact is that no antigenic shift has been observed in this type of influenza viruses.¹⁵

Moreover, influenza B viruses have a lower genetic diversity than type A and they are not classified into antigenically distinct subtypes based on the membrane glycoproteins, that consist of single hemagglutinin (HA) and neuraminidase (NA) type.¹⁶



Finally, even though influenza B viruses undergo antigenic drift, the evolutionary rates of HA gene is slower than those of influenza A strains.¹⁷


Despite the low rate of antigenic changes, since 1983 influenza B viruses evolved into 2 antigenically and genetically different lineages, named Victoria and Yamagata,^{18,19} The Victoria-lineage was isolated in the majority of influenza B cases during the 1980s, while the Yamagata-lineage prevailed in the most part of the world in the late 1990s.²⁰

According to epidemiological surveillance data, in 2001 the Victoria-lineage strains re-emerged in Europe and United States, and since then the 2 lineages and respective sublineages have widespread co-circulated.^{21,22}

The comprehension of evolutionary mechanisms through efficient surveillance systems allows to improve the vaccine composition and to assess the matching between circulating and vaccine strains, that contribute to the effectiveness of influenza vaccines.^{23–25}

In order to define the burden and the nature of evolution among influenza B viruses in Liguria region, Northern Italy, in the 15 y following the re-emergence of Victoria-lineage during the 2001–2002 influenza season, we integrated data deriving from 3 data sources: the epidemiological, virological and syndromic surveillance systems.

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 Supplemental data for this article can be accessed on the [publisher's website](#).

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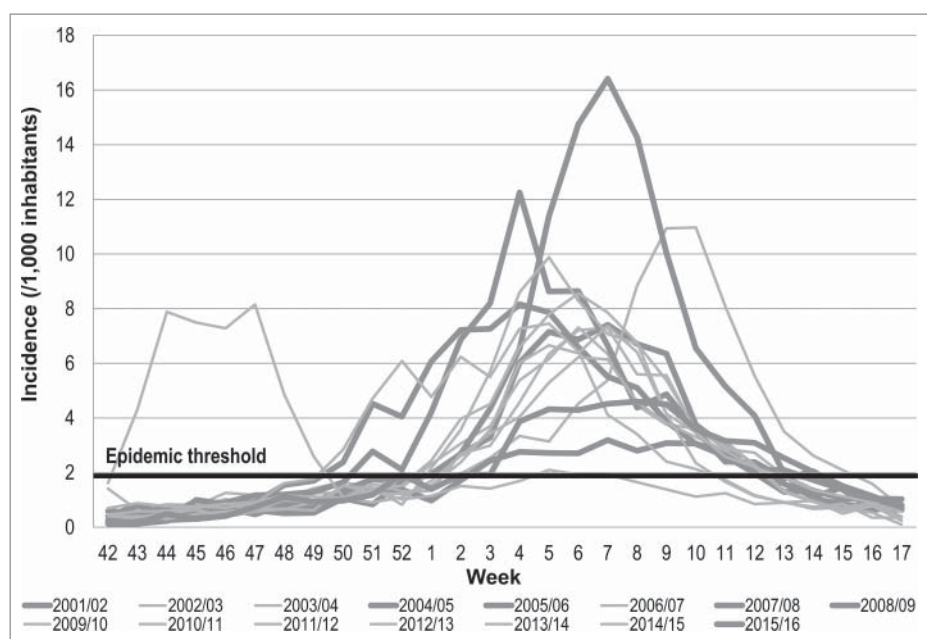


Figure 1. Incidence of Influenza-like illness (ILI) in Liguria Region, Italy, during influenza seasons from 2001/2002 to 2015/2016.

Results

Clinical-epidemiological surveillance

The results of ILI clinical-epidemiological surveillance from 2001 to 2016 are shown in Fig. 1.

Considering the entire study period, it can be estimated that a mean of 56,641 cases of ILI occurred in Liguria region from October to April of each influenza season. However, excluding the 2009 H1N1v pandemic, the number of cases was nearly 53,000. The mean incidence was 2.7 ILI cases per 1,000 inhabitants, ranging from 1.6/1,000 in the season 2005/2006 to 4.1/1,000 in the season 2004/2005.

Among the epidemic periods that were usually observed between the beginning of January and the end of March, the highest and the lowest incidence peaks were 16.4 and 3.2 per 1,000 inhabitants, and they were observed in the weeks 7th/2005 and 7th/2006, respectively. On average, the length of the epidemic was 11 weeks and ranged from 7 to 16 weeks.

The epidemic period, its length in weeks and the estimated number of cases, broken down by age-class, for each influenza season were reported in Supplementary file 1.

Comparing the influenza seasons when the type B influenza virus prevailed with the epidemic when type A was prevalent, the mean incidences of ILI were 2.6 ($\pm 2.5SD$) and 2.7 per 1,000 inhabitants ($\pm 2.7SD$) ($p = 0.86$).

The mean incidence observed when a type B virus mismatch occurred was significantly higher than the incidence estimated when a circulating strains matched with vaccine strains, both not including and including the pandemic season (2.8 vs 2.6 per 1,000 inhabitants, $p < 0.001$).

The median epidemic period did not differ between the seasons when the vaccine mismatch and when no mismatch was observed.

Considering the stratification by age-class, the highest peaks of morbidity were observed in the pediatric age (0–14 y until 2002/2003 and 0–4 and 5–14 subsequently). The incidence rates were usually lowest in the elderly, being appreciable only

in 2001/2002 (peak value of about 2.6 per 1,000), when the type B viruses were prevalent and in 2004/2005 (peak value of about 1.7 per 1,000), when A/H3N2 predominated.

Considering the influenza seasons when a type B vaccine mismatch was observed, the highest peak incidences in the pediatric age were observed during the 2001/2002 and the 2004/2005 season (26 and 9.2 per 1,000 inhabitants). During the other seasons the highest incidence rates were 8.9 and 8.7 per 1,000 inhabitants in 2002/2003 and 2010/2011, respectively. Also the highest incidence rates observed in adults and the elderly were observed when a type B vaccine mismatch was observed, in particular during the 2001/2002 season (6.7 and 2.6 per 1,000, respectively).

Syndromic surveillance

The syndromic surveillance system is active from the season 2006/2007, when no type B influenza virus was detected in Liguria region (Fig. 2). In children, the mean of the epidemic activity indicators in the whole period was 1.65 ($\pm 0.75SD$), with a median duration of 69 d (25–75p: 59–74). The epidemic threshold were commonly exceeded from the end of December to the end of March, with the exception of the pandemic season, when the breakthrough was observed earlier, from the end of September to end of November, 2009. The mean of the activity indicators above the cut-off observed during the seasons when a type B mismatch was observed was 1.5 ($\pm 0.44SD$), lasting a median of 62 d each season. During the seasons when a type B vaccine matching was observed, the mean of the activity indicator was 1.7 ($\pm 0.85SD$), with a median of epidemic period of 71 d for each season ($p < 0.001$). The highest mean activity indicators were observed during the 2007/2008 influenza season, when type B partial mismatch was observed and type A/H3N2 predominated and during the pandemic and the 2010/2011 influenza season, when the A/H1N1 virus prevailed.

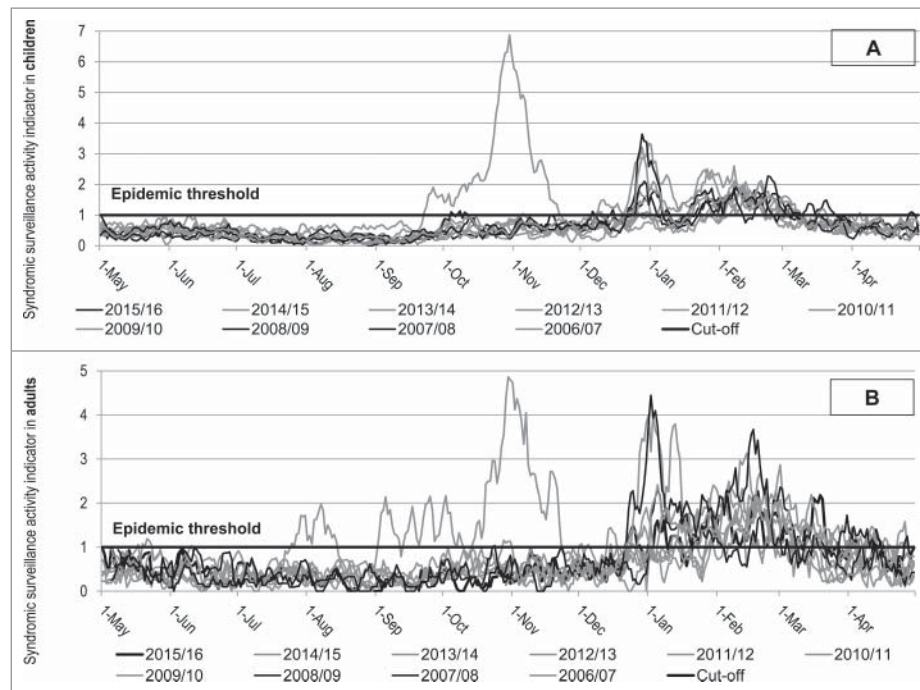


Figure 2. Syndromic surveillance activity indicator for Influenza-like illness (ILI) in children (A) and adults (B) in Liguria Region, Italy, by season.

As regards the ED accesses of adults for ILIs (Fig. 2), the mean of the epidemic activity indicators in the whole period was $1.66 (\pm 0.64SD)$, with a mean duration of 79 d (25–75p: 58–94). The epidemic thresholds were commonly exceeded from the end of December or early January to March–April, with the exception of the 2009/2010 pandemic season, that was characterized by an early and prolonged breakthrough, from August to April. Considering the influenza seasons when a mismatch occurred, the mean of the activity indicators above the cut-off was $1.63 (\pm 0.63 SD)$ and lasted a median of 92 d each season. When a vaccine matching was observed and excluding the pandemic season, the mean of the activity indicator was $1.65 (\pm 0.54 SD)$, with a median of epidemic period of 58 d for each season ($p = 0.66$). As observed in the ED accesses of children, the highest mean activity indicators were observed during the pandemic and the 2010/2011 epidemic seasons, when the A/H1N1 virus predominated.

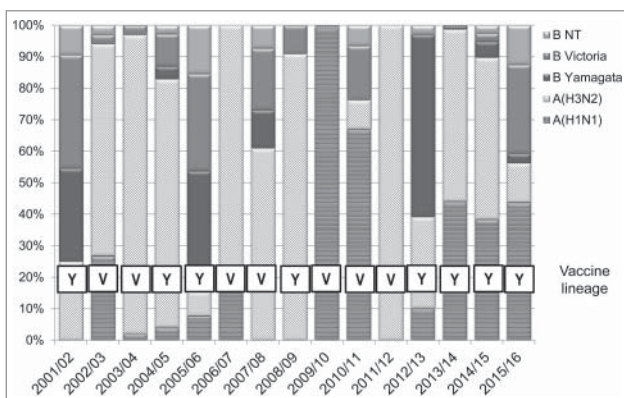


Figure 3. Seasonal distribution of the Ligurian influenza virus positive samples detected from the 2001/2002 to the 2015/2016 influenza seasons.

Virological surveillance

The seasonal distribution of the number of Ligurian influenza virus positive samples detected from the 2001/2002 to the 2015/2016 influenza seasons is reported in Fig. 3.

From the 2001/2002 to the 2015/2016 seasons 1850 influenza viruses positive samples were detected. Among these, 235 (12.7%) were type B viruses. The proportion of isolated type B compare with type A influenza viruses positive samples widely ranged from 0% to 85%. When the lineage was evaluated, the co-circulation of both Yamagata and Victoria lineages was observed in about 40% of the evaluated influenza seasons. Furthermore, during 9 (60%) seasons, the Victoria lineage predominated. A type B vaccine mismatch between circulating and vaccine lineage was observed in 7 (47%) seasons. In particular, during the 2001/2002, 2004/2005, 2005/2006, 2007/2008 seasons the mismatch was partial (between 20% and 59% of the isolated strains belonged to a lineage not included in the trivalent seasonal influenza vaccine) and during the 2009/2010 and the 2015/2016 seasons the vaccine mismatch was complete (> 60% of the isolated strains belonged to a lineage not included in the trivalent seasonal influenza vaccine).

As observed in Tramuto F et al.¹⁵ a lineage swap from the predominance of Victoria to that of Yamagata lineage occurred between 2010/2011 and 2012/2013 seasons.

Antigenic and molecular analysis of influenza B isolates

Antigenic relationships between influenza B virus isolated during the surveilled period and investigated by the HI and/or the NT tests and comparative analyses of the amino acid sequences of the HA1 domains are shown in Supplementary files 2 and 3.

Most of the HAs of the B isolates of the 2001/2002 season cluster in distinct clades of the Victoria- and the Yamagata-

lineages. HAs within the Yamagata-lineage showed high identity to the HA of the B/Sichuan/379/99 and B/Harbin/7/94 strains and differed by 2 amino acid changes (L58F and N126D). HAs of viruses belonging to the Victoria lineage were similar to the vaccine strain B/HongKong/330/2001, with the exception of the amino acid substitution T199I.

Isolates Victoria-like of the 2002/2003 and 2004/2005 seasons showed highly similar NT titres and amino acidic sequences compare with B/Shandong/07/1997. Considering the Yamagata-like strains that circulated during the 2004/2005, 2005/2006, 2007/2008 seasons, high matching was found with the B/Jangsu/10/2003 virus, with the exception of 4 amino acidic changes.

From 2005 to 2010, circulating viruses Victoria-like showed the same sequence amino acidic of the vaccine strain B/Malaysia/2506/2004, with the exception of the 2008/2009 influenza season, when viruses B/Brisbane/60/2008-like were found both from a serologic and molecular point of view. Viruses belonging to the same lineage and circulating in the following seasons were B/Brisbane/60/2008-like, too and only one amino acidic substitution was found (I146V).

As shown in the phylogenetic tree (Fig. 4), the Yamagata-like viruses isolated during the 2012/2013 season were B/Wisconsin/01/10-like, even if 8 amino acidic substitutions were found. The following seasons were characterized by viruses B/Massachusetts/02/12-like and B/Phuket/3073/2013-like. Of note, the virus B/Genoa/01/2014 showed distinguished antigenic and molecular pattern.

Finally, the phylogenetic tree is noticeably composed of 2 main lineages, the Victoria- and the Yamagata-lineages. A gradual drift was observed in both lineages, allowing to identify further subgroups into each one.

The Victoria-lineage include B/HongKong/330/0, B/Shandong/7/97, B/Malaysia/2506/2004 and B/Brisbane/60/2008 genetic clades, whereas within the Yamagata-lineage there are 4 branches represented by B/Sichuan/379/99, B/Massachusetts/02/2012 B/Wisconsin/01/10 and B/Phuket/3073/2013.

Discussion

The estimation of public health impact of the influenza epidemics is challenging. Etiologic agents of ILIs are numerous, the probability of contact with GPs varies by age,²⁶ and even integrated surveillance systems such as those active in Liguria region don't allow conclusive evaluations except for particular epidemiologic conditions.

However, this 15-years study allowed to better define the epidemiology and the clinical burden of influenza viruses, with a particular focus on type B viruses, in the Liguria Region of Italy.

From the epidemiologic standpoint, both clinical-epidemiological and syndromic surveillances highlighted the heavy burden of ILI syndrome particularly among children: as reported, the age group with the highest peak incidence in all seasons was that of subjects aged < 14 y. The incidence was relevant also among adults and elderly, even though the pronounced variability observed among seasons.

Syndromic surveillance further confirmed the impact of ILI in terms of ED accesses, revealing breaking through the epidemic threshold during all influenza seasons and particularly

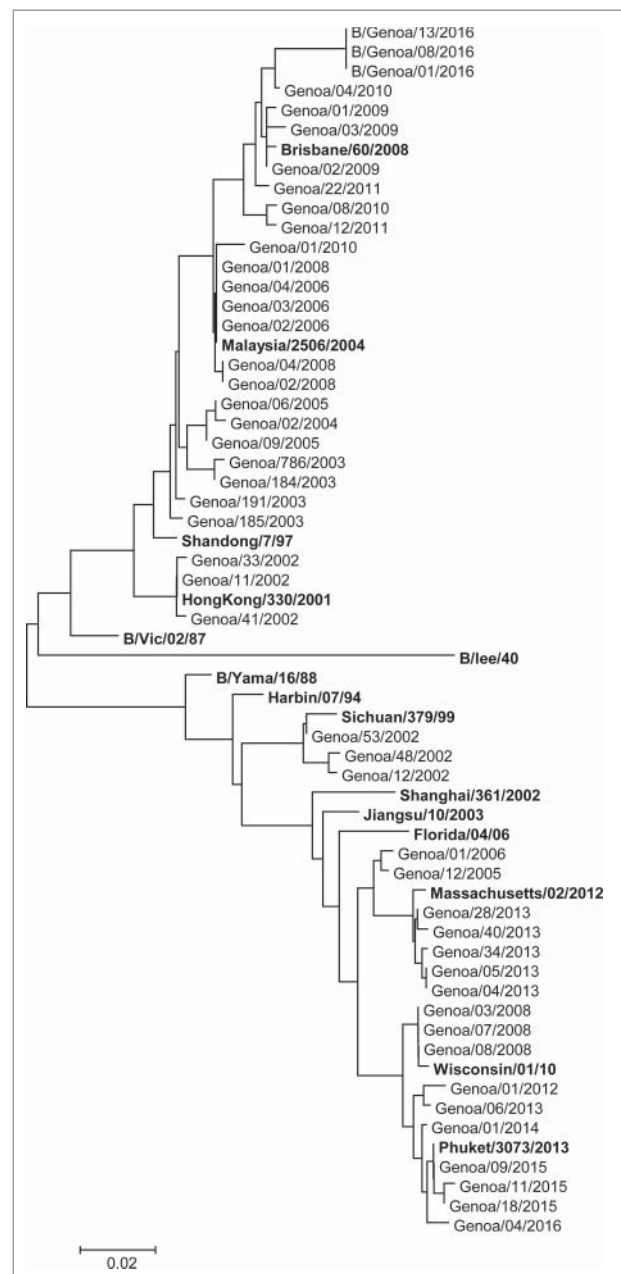


Figure 4. Phylogenetic analysis of the subunit 1 of the Hemoagglutinin (HA1) nucleotide sequences from influenza B Yamagata-land Victoria-lineages isolated in Liguria region, Italy, from 2001/02 to 2015/16 influenza seasons.

in the period from the end of December to the end of March characterized by sustained circulation of influenza viruses.

In this context, the virologic surveillance evidenced the heavy impact of influenza B viruses that resulted the predominant cause of influenza in 3 of 15 seasons and determined a relevant number of influenza cases in further 4 seasons.

Moreover, based on sequence analysis data, we assisted to an heterogeneous circulation of different influenza B strains during the surveillance period.

In particular, the genetically characterization of the influenza B viruses demonstrated the co-circulation of both B lineages in the majority of the evaluated seasons and allowed to recognize a partial or complete mismatch with type B vaccine strain in at least 6 seasons. In particular, we registered a

complete vaccine mismatch in 2008/2009 and 2015/2016 seasons, because of the circulation of viruses belonging to the Victoria-lineage, while the vaccine strains belonged to the Yamagata-lineage. The co-circulation of both lineage and the subsequent partial mismatch was observed during 4 influenza seasons (namely 2001/2002, 2004/2005, 2005/2006 and in 2007/2008).

This regional virologic scenario was coherent with the Italian one,²⁷ substantially differing from that observed in other European countries and in US where a complete type B mismatch was registered more frequently (Supplementary file 4).²⁸

During 2001/2002 season, Victoria-lineage, Hong Kong-like viruses appeared on Italian and global scene, with an heavy burden on Italian population, in which an high rate of susceptible individuals was present, due to the lack of circulation of this lineage during the 1990s.²⁹ However, Yamagata-lineage viruses were not replaced by Victoria-like viruses, determining a co-circulation of both lineages, event already observed during previous seasons.^{30,31}

Since 2002/2003 influenza season, reassortant B viruses, possessing a Victoria-lineage HA and Yamagata-lineage NA, were isolated more and more frequently, but they showed not to be the result of reassortment between the co-circulating strains during 2001/2002 season in Italy, as described previously by Puzelli et al.²³ These mixed strains were the results of previous recombinant event between co-circulating B viruses occurred outside Italy.^{32,33}

After a prevalent circulation of Victoria-lineage viruses in the period 2009–2012, since 2012–2013 Yamagata-lineage viruses predominated over Victoria-lineage strains.¹⁵ During the 2015/16 season, Victoria-lineage Brisbane/08-like viruses prevailed again both at global and local level. This picture led to the new recommendations for the influenza trivalent vaccine composition for the season 2016/2017 including Victoria-lineage Brisbane/08 virus in replacement of Yamagata-lineage Phuket/13 virus.

Noteworthy, the partial or complete mismatch between circulating and vaccine type B strains resulted associated with a higher incidence of ILI in all age groups and with a higher incidence of ED accesses as well as with a longer epidemic period among children and adolescents. The predominant role of influenza B viruses among these latter age-groups is in line with other international data.³⁴

The antigenic analysis confirmed the high variability of circulating influenza B virus and allowed to estimate the antigenic distance between the vaccine candidates and the circulating strains of influenza virus. Our results indicated a frequent co-circulation of influenza B viruses closely related to the vaccine strains and the appreciable proportion of viruses presenting lower reactivity with the reference serum. Importantly, HI assay results highlighted the high antigenic distance between viruses belonging to the 2 lineages.

Combined serologic and molecular analyses are useful to reveal the major changes in antigenicity and to better evaluate the characteristics of any new or re-emerging influenza B viruses, in order to allow the best choice of the viral strain to include in the vaccine composition.

The high proportion of type B isolates observed during the last 15 y in the majority of European and Extra-European countries and the high frequency of vaccine mismatch, that potentially impaired the vaccine effectiveness, arose the

scientific debate about the opportunity to include both lineages of type B influenza virus in the seasonal influenza vaccine to improve the protection against type B viruses.³⁵

The study had some major limits regarding the methodology of epidemiologic surveillance that varied during the study period, with the introduction of syndromic surveillance since 2006/2007 season. A further limit was represented by the lack of demographic and clinic data about the patients affected by ILI.

Moreover, the number of samples collected for the virologic surveillance and their sources were heterogeneous and varied among seasons; antigenic and molecular analysis were performed only on a small proportion of samples, even though representative of the circulating strains.

In conclusion, Influenza viruses type B cause significant burden in terms of morbidity and ED accesses, with variable incidence in the considered period.

In the majority of the observed influenza seasons, the co-circulation of 2 antigenically distinct lineages was observed and no cross-reactivity between lineages has been detected.

Both the unpredictability of predominant lineage and the subsequent mismatch between circulating and vaccine strains and the heterogeneity of circulating sublineage may determine reduced vaccine effectiveness.

The integration of epidemiological, syndromic and virological (molecular and serological) surveillance systems allow to monitor the burden of influenza and to follow the genetic evolutionary pattern and antigenic variability of viral strains and to find variants that will probably circulate in the coming season and include them in the vaccine formulation.

The recent introduction of quadrivalent influenza vaccine would more accurately reflect the current epidemiology of influenza and would improve vaccine effectiveness, optimizing the control of this public health threat.

Materials and methods

The clinical-epidemiological surveillance system

The Liguria region contributes to the national surveillance of influenza-like illness (ILI) instituted by the National Institute of Health (NIH). In particular, the clinical and epidemiological surveillance system is performed by a network of sentinel general practitioners (GPs) who cover 2% of the regional population (meanly 50,443 patients per year) and notify cases of ILIs from week 42th to week 17th of each influenza season.

ILIs cases are defined as acute onset of fever together with respiratory symptoms and one systemic symptom such as headache, general discomfort, asthenia, myalgia, according to the Italian surveillance network guideline.³⁶ A standardized report form, including demographic and clinical information is used for notification.³⁶

The reports of ILIs cases diagnosed in Liguria region are sent to the inter-University Center for Research on Influenza and other Transmissible Infections (CIRI-IT), Genoa (Italy), that is one of the 2 reference centers of the Italian epidemiological surveillance system of influenza (Influnet). Regional data are weekly sent to the NIH, that collects and elaborates epidemiological information at the national level.^{36,37}

The syndromic surveillance system

The ILI syndromes were monitored through the Syndromic surveillance system (SSS) based on Emergency Department (ED) accesses. This SSS is active since 2006 and evaluate data collected at the Ligurian reference university hospital for adults “San Martino,” that covers approximately 55% of all catchment area in Genoa, the regional capital city.^{38,39}

Since 2007 the regional SSS collects data from the EDs of another main hospital for adults and the regional reference hospital for children in Genoa, allowing to cover 72% and 100% of all urban area ED accesses for adults and children, respectively.

Syndrome coding, data capture, transmission and processing, statistical analysis to assess indicators of disease activity and alert thresholds and signal response were operatively described in Ansaldi et al.³⁸

The number of ED accesses and incidence of accesses for ILIs in the considered period were stratified by age and influenza season. Further analysis about the incidence of ILIs during seasons when vaccine matching and mismatching occurred were conducted.

The virological surveillance system

Nasopharyngeal swabs were collected using Virocult swabs (MWE, Medical Wire, Corsham, UK) from patients with ILI syndrome who accessed to the abovementioned hospitals or visited by the sentinel GPs during the influenza epidemic season and then sent to the Regional Reference Laboratory for influenza surveillance at the Department of Health Sciences, University of Genoa for influenza virus characterization.

The characterization was also conducted by the National Influenza Center at the NIH and/or by the World Health Organization (WHO) Influenza Collaborating Center in London (UK), which participate in the WHO Influenza Surveillance Network.

All influenza positive samples were tested for type A and B influenza viruses,¹⁹ and a representative subset was further subjected to an antigenic and/or genetic characterization.

The antigenic characterization was performed by hemagglutination inhibition (HI) and/or microneutralisation (NT) tests, in order to identify the antigenic variants circulating in human populations during the winter season.

The HI test was performed using whole viruses and hyperimmune sheep serum or post-infection ferret sera to reference viruses (provided by WHO Influenza Collaborating Center, London, UK) as described by Puzelli et al.,²³ and by Ansaldi et al.²⁹

The microneutralisation assay was performed as reported by Ansaldi et al.⁴⁰

Furthermore, since the antigenic variability of an influenza virus HA mainly occurs in the HA1 domain,⁴¹ we determined the nucleotide sequences encoding the HA1 subunit of selected influenza B viruses circulating in Liguria during the last 15 influenza seasons, as previously described.²⁹

Finally, the construction of phylogenetic trees was performed applying the Kimura-2 distance method and the Neighbor-Joining algorithm with 1000 bootstrap replicates using the MEGA v5.05 software package.⁴²

Abbreviations

| | |
|----------|--|
| ILI | Influenza-like illness |
| HA | Hemagglutinin |
| NA | Neuraminidase |
| NIH | National Institute of Health |
| GP | General Practitioner |
| CIRI-IT | Inter-University Center for Research on Influenza and other Transmissible Infections |
| Influnet | Italian epidemiological surveillance system of influenza |
| SSS | Syndromic surveillance system |
| ED | Emergency Department |
| WHO | World Health Organization |
| HI | Hemagglutination inhibition |
| NT | Microneutralization. |



Disclosure of potential conflicts of interest

Filippo Ansaldi, Giancarlo Icardi and Laura Sticchi have previously participated at speaker's bureaus and advisory board meetings sponsored by GSK, Pfizer, Novartis and Sanofi Pasteur. Cecilia Trucchi, Cristiano Alicino, Chiara Paganino and Andrea Orsi Ilaria Barberis, Federico Grammatico, Paola Canepa, Emanuela Rappazzo, Bianca Bruzzonedeclare that they have no conflict of interest.

Funding

Filippo Ansaldi, Giancarlo Icardi and Laura Sticchi received research funding as investigator or principal investigator from GSK, Pfizer, Novartis and Sanofi Pasteur MSD.

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