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ORIGINAL RESEARCH

Moderate influenza vaccine effectiveness against A(H1N1)pdm09 virus, and low effectiveness against A(H3N2) subtype, 2018/19 season in Italy

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ABSTRACT

Background: Influenza vaccines are updated every year to match the vaccine strains with currently circulating viruses; consequently influenza vaccine effectiveness (IVE) has to be assessed annually.

Research design and methods: A test-negative case-control study was conducted within the context of the Italian sentinel influenza surveillance network to estimate IVE by age group, virus subtype, and vaccine brand in medically attended laboratory-confirmed influenza.

Results: In Italy, the 2018/19 influenza season was characterized by the co-circulation of influenza A (H1N1)pdm09 and A(H3N2) viruses. The adjusted IVE estimate in preventing influenza was moderate (44.8%, 95% CI: 18.8 to 62.5) against A(H1N1)pdm09, whereas there was no evidence of effectiveness (1.8%, 95% CI: −37.8 to 30.1) in persons affected by A(H3N2). IVE against A(H1N1)pdm09 decreased with age ranging from 65.7% to 13.1% among children/adolescents and elderly, respectively; moreover results suggest that Vaxigrip Tetra® was more effective against A(H1N1)pdm09 compared to Fluarix Tetra® [62.5% (95% CI: 34.3 to 78.6) vs 24.5% (95% CI: −40.6 to 59.6)]. Low effectiveness (35.2%, 95% CI: −50.8 to 72.1) against A(H3N2) was detected only in the elderly immunized with Fluad®.

Conclusions: Findings suggest that influenza vaccines were low to moderately effective, probably due to a mismatch between circulating and vaccine strains.

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Influenza vaccine effectiveness; test-negative case-control study; national influenza surveillance network; Italy

1. Introduction

Vaccines developed to protect against seasonal influenza illness are updated annually to match the vaccine strains with currently circulating viruses [1]. Indeed, since influenza viruses change rapidly due to antigenic drift, vaccines are regularly reformulated and delivered through seasonal campaigns. Since influenza vaccination is a public health intervention, it is important to evaluate its effectiveness every year. Licensed vaccines include inactivated or live-attenuated influenza type A and B viruses, with two subtypes of A virus and one or two lineages for B virus [2]. Specifically, trivalent influenza vaccines contain two A virus strains (H1N1 and H3N2) and one B virus strain, while quadrivalent vaccines include an additional B virus lineage.

The World Health Organization (WHO) provides recommendations on which viruses will be included in vaccine composition for the forthcoming influenza season; however, it does not give any preferential recommendation for influenza vaccine products for target population for whom more than one has been licensed. However, not all registered influenza vaccines are available every year and fluctuations in production and distribution often limit the options. Observed influenza vaccine effectiveness (IVE) varies by seasons due to a variety of reasons including disease severity level, a mismatch between the vaccine virus and circulating strains, type of vaccine used, immunized population, and possible interference from previous vaccinations [3,4]. Therefore, there is a need for more studies focusing on comparing the effects of vaccination by virus subtype, vaccine type, and

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Article highlights

- In the 2018/19 influenza season in Italy, results suggest that vaccines were low to moderately effective, with estimates varying depending on the patients' age, virus subtype, and vaccine brand.
- Quadrivalent vaccines conferred moderate effectiveness in preventing influenza caused by A(H1N1)pdm09, while no evidence of protection against A(H3N2) was found.
- Good effectiveness against A(H1N1)pdm09 was detected only in children/adolescents and in subjects immunized with Vaxigrip Tetra®, whereas a mild benefit against infection caused by A(H3N2) was found only among individuals who received Fluad®.
- Findings suggest a moderate IVE against A(H1N1)pdm09 and low or no IVE against A(H3N2) virus, probably due to a mismatch between circulating and vaccine strains.

vaccine brand, according to the new European Medicines Agency guideline on influenza vaccines [5].

In order to provide IVE estimates in the 2018/19 influenza season in Italy, a test-negative case-control study was conducted within the context of the national sentinel influenza surveillance network (InfluNet), coordinated by the National Institute of Health [Istituto Superiore di Sanità (ISS)], and based on sentinel General Practitioners (GP) and Regional Reference Laboratories (RRL) present throughout the country. The estimates obtained include effectiveness against medically attended laboratory-confirmed influenza adjusted for the main confounding variable [6].

2. Patients and methods

2.1. Study population

In Italy, the National Influenza Surveillance has been established to ensure the early detection of influenza epidemics for public health prevention and control activities, to estimate the impact of the epidemic, to characterize the circulating viruses for vaccine virus selection, and to assess the effectiveness of influenza vaccines. To these aims, clinical samples coming from GPs and hospitals are collected annually and analyzed by RRL for diagnosis of influenza using a real-time RT-PCR assay. Furthermore, molecular characterization and phylogenetic analysis of the hemagglutinin (HA) genes are performed on a subset of the circulating influenza viruses by the National Influenza Centre (NIC), located at ISS, with the objective to evaluate how circulating strains match with those included in the influenza vaccines for the ongoing season, and to detect possible emerging genetic variants. The Neighbor-Joining method available in Mega 6.0 software (http://www.megasoftware.net) [7], and HA sequences from reference strains retrieved from the EpiFlu database of the GISAID (Global Initiative on Sharing All Influenza Data) [8] were used for the analyses.

The Influenza Sentinel Surveillance Network (InfluNet) involves nearly 1,000 GPs covering about 2.3% of the Italian population. However, in addition to the epidemiological surveillance (in which only aggregated data are collected weekly, and ILI cases are not laboratory-confirmed), a subset of GPs also participate voluntarily to the virological surveillance of influenza circulating viruses aimed at evaluating the IVE annually [9]. The target groups for influenza vaccination in Italy are the followings: people ≥65 years old, individuals aged 6 months-64 years with specific chronic conditions, pregnant women in the second or third trimester of pregnancy, people working in public services, and healthcare workers [10].

2.2. Study design

A test-negative case-control study was conducted within the InfluNet surveillance system. The study population consisted of patients consulting a participating GP for an influenza-like illness (ILI), defined according to the European Union case definition [11]. A systematic sampling of the first 2 ILI patients <65 years old that presented each week was used, whereas all patients ≥65 years with ILI were sampled. A case of influenza was defined as an ILI patient who was swabbed within 7 days from symptoms onset and tested positive for influenza virus, whereas controls were ILI patients who tested negative.

A subject was considered vaccinated if he received the influenza vaccine more than 14 days from the ILI symptoms onset. The GP interviewed the ILI patients using an on-line standardized questionnaire, to collect both demographic (age and sex) and clinical information, including date of symptoms onset, vaccination status, vaccination date, vaccine brand, influenza vaccination in any of the previous two seasons, presence of underlying chronic conditions (including diabetes, pulmonary and cardiovascular diseases, renal disease, cancer, immunodeficiency), number of practitioner visits and number of hospitalizations due to chronic conditions in the last year.

GP captured vaccination date and vaccine brand through a process in which data were initially recorded on paper and subsequently manually entered into a database. Vaccinated subjects were identified only by GP medical records, and patients did not receive any vaccination card.

The study period started at week 42–2018 (15th of October) and ended at week 17–2019 (28th of April). All subjects gave their informed consent to participate in the study, which was approved by the ISS Ethics Committee on the 23rd of November 2018.

2.3. Sample size

A case-control ratio of 1:1 was used for sample size consideration; assuming 80% power and two-sided 95% confidence levels, with a vaccination coverage of 5% for individuals aged 6 months–64 years, and 50% for elderly ≥65 years old. In this setting, 1,140 subjects (570 cases and 570 controls) were needed in each of the two younger age groups (6 months–17 years or 18–64 years) to detect at least 62% IVE as statistically significant; 280 subjects (140 cases and 140 controls) aged ≥65 years were needed to detect at least 50% IVE as statistically significant. The overall reached sample size was 2,526 in line with the expected value (2,560): 1,250, 1,082 and 194 in 6 months-17 years, 18–64 years and ≥65 years old respectively.

2.4. Statistical analysis

The primary objective was to estimate overall and age-specific (6 months-17 years old, 18–64 and ≥65 years old) seasonal IVE

against medically attended laboratory-confirmed influenza. The definition of the age groups was based on the approved indications and recommendations of the commercial vaccines. Crude and confounder-adjusted overall IVE were estimated as (1-Odds Ratio) x100 by univariable and multivariable logistic regression models. The following confounding variables, as suggested by European Medicines Agency (EMA) and European Center for Disease Prevention and Control (ECDC) [5,6], were considered: age (modeled as categorical variable: 0–2, 3–4, 5–9, 10–19, 20–29, 30–39, 40–49, 50–59, 60–69 and ≥70 years old), sex, presence of chronic conditions, date of ILI symptoms onset (modeled as a restricted cubic spline with four knots), number of GP consultations (0, 1–5, >5) in the previous year in order to document and control for access to care, hospitalizations (0, ≥1) due to chronic conditions in the previous 12 months (proxy of severity of the underlying conditions), influenza vaccination in any of the previous two seasons. Missing values were present for the number of GP visits, number of hospitalizations and previous vaccinations (ranging from 14% to 4%). However, results from preliminary multivariable analyses performed in a subset of data without missing values suggested that they did not affect the IVE estimates and did not add any improvement in the goodness of fit of the model; therefore, these variables were excluded from the analyses in the final models. The secondary objective was to estimate IVE by influenza virus subtype (A(H1N1), A (H3N2)), and vaccine brand (Vaxigrip Tetra®, Fluarix Tetra®, Fluad®) [5]. To evaluate IVE by age group, by virus subtype, and by vaccine brand, models including an interaction term between vaccination status and age group, virus subtype, and vaccine brand were performed.

Statistical analysis was carried out using the Stata software, version 15 (Stata Cooperation, College Station, Texas, USA).

3. Results

3.1. The 2018/19 influenza season in Italy: epidemiological and virological characteristics

The influenza epidemic (according to the Moving Epidemic Method developed by ECDC *[12]*), started at week 49–2018 (3rd–9th of December) and ended at week 12–2019 (18th–24th of March). Considering the epidemiological surveillance based on sentinel GPs, a total of 186,564 ILI cases (with a diagnosis of influenza not laboratory-confirmed) were collected, coming from a population of about 1,366,788. Therefore, reproportioning at the national level, the estimated number of ILI cases was around 8 million, indicating a high level of intensity for 2018/19 influenza season. The peak of incidence rates of ILI cases (14 per 1,000 population) was reached in week 5–2019 (28th of January-3rd of February) (Figure 1(a)).

For virological surveillance, a total of 20,174 clinical samples were collected at the national level and tested for the influenza virus; 6,401 (31.7%) of them were positive for influenza virus, showing a peak (47.1%) at week 6–2019 (4th–10th of February) (Figure 1(a)). The 2018/19 influenza season was dominated by type A viruses and characterized by the co-circulation of A (H1N1)pdm09 and A(H3N2) subtypes (Figure 1(b)). Specifically, 6,392 (99.9%) were positive for influenza A viruses and 9 (0.1%) for influenza B viruses. Among 5,924 subtyped influenza A viruses, 2,969 (50.1%) were A(H1N1)pdm09 and 2,955 (49.9%) were A(H3N2). Genetic analyses performed on 178 (6%) of the A(H1N1)pdm09 viruses showed that they clustered within the 6B.1A subclade, defined by the HA amino acid substitutions S74R, S164T and I295V as compared to the vaccine virus strain A/Michigan/45/2015 (6B.1 clade). The majority (88.7%) of the sequenced viruses carried also the amino acid substitution S183P, often with additional substitutions in the HA including T185I and N129D. Genetic characterization of 276 (9.3%) A(H3N2) viruses highlighted that 195 (70.6%) fell in subclade 3C.2a1b, 15 (5.4%) in subclade 3C.2a2, and 66 (24.0%) in clade 3C.3a.

3.2. Test-negative case-control study

The number of sentinel GPs participating in InfluNet, taking throat swabs from ILI patients, was 245 covering 316,237 patients; the Italian population coverage was about 0.5%.

Within the test-negative case-control study, 2,655 individuals were enrolled, but in order to estimate seasonal IVE against laboratory-confirmed influenza in a primary care setting, 2,526 subjects were evaluated (Figure 2). The reasons and the frequency to be excluded from this analysis were the followings: 18 patients were outside the influenza season window (only controls were detected), 11 were <6 months of age, 39 were swabbed >7 days after ILI symptoms onset, 17 were partially vaccinated (vaccine administration ≤14 days

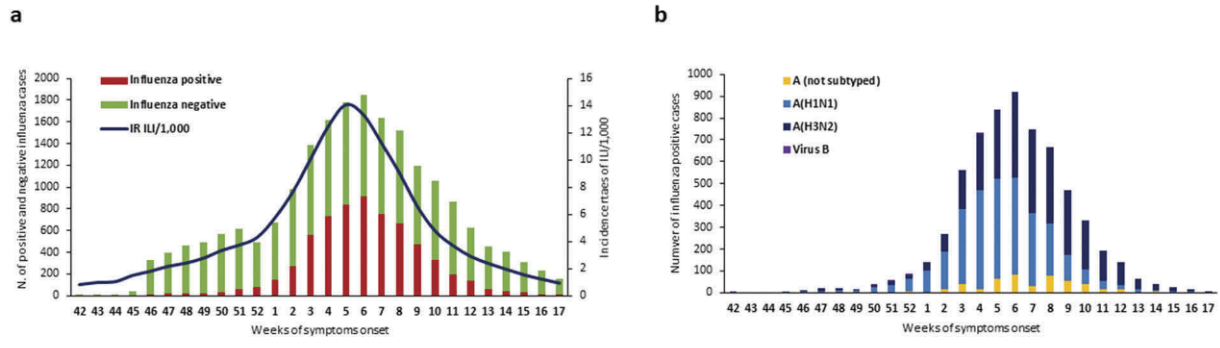

Figure 1. Incidence rates of patients presenting with influenza-like illness by week of symptoms onset, and number of positive and negative influenza cases (Panel a); influenza cases by virus subtypes (Panel b).

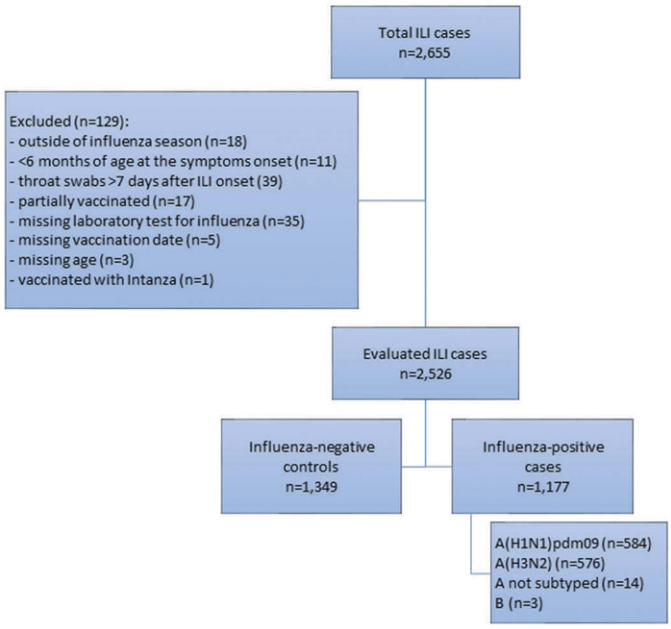

Figure 2. Study flow-chart of the test-negative case-control study.

before symptoms onset), 43 had missing data (laboratory test result, vaccination date, age), and 1 was immunized abroad with a vaccine not licensed in Italy. Among 2,526 cases tested for influenza virus, 1,177 were positive (46.6%). The highest proportion of influenza cases was reported in the 6 months-17 years age group (50.3%), followed by the 18–64 years (42.2%), and the ≥65 years old (40.7%). The percentage of positive tests were similar among GPs, since they have been well trained and most of them have been participating in the surveillance network for several years.

Among all enrolled subjects, only 8% were elderly; indeed, ILI cases are usually less common in the elderly than in other age groups, since vaccination uptake is much higher in subjects ≥65 years old as compared to children and adults. This was the reason for the systematic sampling of the first 2 ILI patients <65 years old that presented each week, and of all patients ≥65 years.

Most patients aged 18–64 years were infected by A(H1N1) pdm09 virus (58.5%), whereas A(H3N2) type virus was predominant among subjects aged 6 months-17 years and ≥65 years old (54.5% and 59.0%, respectively). The proportion of vaccinated subjects was 13.2%, 7.4% among patients aged 6-months-17 years, 10.4% among those aged 18–64 years, and 66.5% among people ≥65 years old. For the 2018/19 season, three vaccine types were available in Italy, trivalent inactivated vaccine (TIV), adjuvanted TIV (aTIV) and quadrivalent inactivated vaccine (QIV) (Table 1). Most subjects received QIV (80.7%), followed by aTIV (18.4%) and TIV (0.9%). The median age of subjects who were immunized with QIVs (64.1% with Vaxigrip Tetra®, 35.9% with Fluarix Tetra®) and aTIV was 49 and 75 years, respectively.

Participants profiles included in the IVE analysis are displayed in Table 2 for all influenza A cases (n = 1,174), A(H1N1)pdm09 cases (n = 584), A(H3N2) cases (n = 576), and test-negative controls (n = 1,349). Positive and negative influenza subjects were similar for almost all the considered variables: 53% were males, <9% were aged ≥65 years, 31% had chronic conditions, <4% were hospitalized in the previous year, and 10% were vaccinated in any of the previous two seasons. However, controls were older (p = 0.001) and had more GP visits in the previous 12 months (p = 0.024) than cases.

3.3. Influenza vaccine effectiveness

After adjustment for all relevant covariates, vaccine effectiveness was estimated to be 25.0% [95% confidence interval (CI): 0.4 to 43.6] against illness caused by influenza A viruses, 44.8% (95% CI: 18.8 to 62.5) against A(H1N1)pdm09 and 1.8% (95% CI: −37.8 to 30.1) against A(H3N2) subtype (Table 3 and Figure 3(a)). Vaccine effectiveness against A(H1N1)pdm09 decreased with age, as 65.7% (95% CI: 27.5 to 83.8) was found among children/adolescents aged 6 months-17 years, 37.1% (95% CI: −8.2 to 63.4) among adults aged 18–64 years, and 13.1% (95% CI: −105.2 to 63.2) among elderly aged ≥65 years; a low effect against A(H3N2) virus subtype was found only among adults (11.4%, 95% CI: −50.9 to 47.9). QIVs and aTIV showed similar effectiveness in preventing influenza caused by A viruses (22.6% and 21.4% respectively), however among QIV Vaxigrip Tetra® presented a higher value (33.7%, 95 CI: 2.5 to 53.6) compared to Fluarix Tetra® (3.8%, 95% CI: −50.7 to 38.6), particularly against A(H1N1)pdm09 (62.5% vs 24.5%) (Table 3 and Figure 3(b)). No evidence of protection was detected in preventing influenza caused by A(H3N2) subtype using both Vaxigrip Tetra® and Fluarix Tetra®, conversely low effectiveness was found with Fluad® (35.2%, 95% CI: −50.8 to 72.1). Overall, higher values of IVE were found with Vaxigrip Tetra® compared to Fluarix Tetra® both among children/adolescents (43.5% vs 7.9%) and adults (40.0% vs −11.9%); of note very low values of IVE were observed with both QIVs (3.1%) and aTIV (−1.1%) among elderly.

4. Discussion

Vaccination remains the best approach for preventing influenza and its potentially serious complications, including those that

Table 1. Influenza vaccines available in Italy for 2018/19 influenza season.

Brand name	Manufacturer	Type of vaccine	Technical specifications	Approved indication	Number of vaccinees
Vaxigrip Tetra	Sanofi Pasteur	QIV	Split-virion	≥6 months	166 (51.7%)
Fluarix Tetra	GlaxoSmithKline	QIV	Split-virion	≥6 months	93 (29.0%)
Fluad	Seqirus	aTIV	Subunit	≥65 years	59 (18.4%)
Agrippal S1	Seqirus	TIV	Subunit	≥6 months	2 (0.6%)
Influpozzi Subunit	Seqirus	TIV	Subunit	≥6 months	1 (0.3%)

QIV, quadrivalent inactivated vaccine; TIV, trivalent inactivated vaccine, aTIV, MF59-adjuvanted TIV.

Table 2. Characteristics of confirmed cases and corresponding test-negative controls included in the study, 2018/19 influenza season in Italy.

	Cases						Controls		
	Type A		Subtype A (H1N1)		Subtype A (H3N2)		Influenza negative		*Cases vs Controls*
Demographics and clinical characteristics	n	%	n	%	n	%	n	%	*p-value*
All	1,174		584		576		1,349		
Age groups									
6 months-17 years	628	53.5	283	48.5	339	58.8	620	46.0	*0.001*
18–64 years	467	39.8	269	46.0	191	33.2	614	45.5	
≥65 years	79	6.7	32	5.5	46	8.0	115	8.5	
Sex									
Male	627	53.4	306	52.4	316	54.9	722	53.5	*0.954*
Female	547	46.6	278	47.6	260	45.1	627	46.5	
Underlying chronic conditions									
Yes	372	31.7	153	26.2	215	37.3	411	30.5	*0.509*
No	802	68.3	431	73.8	361	62.7	938	69.5	
Number of GP consultations in the last year									
0	194	17.5	107	19.3	86	16.0	219	16.8	*0.024*
1–5	797	72.1	393	70.9	393	72.9	898	69.1	
>5	115	10.4	54	9.8	60	11.1	183	14.1	
Number of hospitalizations for chronic conditions in the last year									
0	956	97.2	493	97.4	450	96.8	1,149	96.3	*0.274*
≥1	28	2.8	13	2.6	15	3.2	44	3.7	
Influenza vaccination in any of the previous two seasons									
Yes	118	10.4	44	7.8	73	13.1	135	10.4	*0.958*
No	1,013	89.6	517	92.2	484	86.9	1,167	89.6	
Influenza vaccination in the current season									
Yes	147	12.5	50	8.6	96	16.7	187	13.9	*0.322*
No	1,027	87.5	534	91.4	480	83.3	1,162	86.1	
Type of influenza vaccine									
QIV	118	82.5	34	70.8	84	89.4	141	79.2	*0.734*
aTIV	24	16.8	13	27.1	10	10.6	35	19.7	
TIV	1	0.7	1	2.1	0	0.0	2	1.1	
Vaccine brand									
Vaxigrip Tetra (QIV)	69	48.2	18	37.5	51	54.3	97	54.5	*0.370*
Fluarix Tetra (QIV)	49	34.3	16	33.3	33	35.1	44	24.7	
Fluad (aTIV)	24	16.8	13	27.1	10	10.6	35	19.6	
Agrippal S1 (TIV)	1	0.7	1	2.1	0	0.0	1	0.6	
Influpozzi Subunit (TIV)	0	0.0	0	0.0	0	0.0	1	0.6	

Chi-squared test was used to compare cases infected by influenza A viruses versus controls.
QIV, quadrivalent inactivated vaccine; TIV, trivalent inactivated vaccine, aTIV, adjuvanted TIV.

may result in hospitalization and death. However, the effectiveness of seasonal influenza vaccine varies by season. Indeed, it could be affected by several reasons including disease severity level, the mismatch between the vaccine and the predominant circulating virus strains, type of vaccine used, immunized population. In particular, during seasons when the influenza vaccine is not well matched to circulating influenza viruses, little or no benefit from vaccination may be observed.

In the 2018/19 influenza season in Italy, results suggest that vaccines were moderately effective, with estimates varying depending on the patients' age, virus subtype, and vaccine brand. Overall, QIV conferred moderate protection against A(H1N1)pdm09 (49.1%) while lack of protection against A(H3N2) was found. Good effectiveness was detected only against A(H1N1)pdm09 in children/adolescents (65.7%) and in subjects immunized with Vaxigrip Tetra® (62.5%), whereas a mild benefit against infection caused by A(H3N2) was found only among individuals who received Fluad® (35.2%). Nevertheless, even though vaccination does not always prevent influenza illness, it could drastically reduce the risk of influenza-associated hospitalizations, protecting against serious medical events associated with several chronic conditions, particularly in the elderly **[13]**.

Overall, the reason for the observed difference in IVE between Vaxigrip Tetra® and Fluarix Tetra® remains unclear. Based on drug technical sheets, both vaccines had the influenza A and B virus strains recommended for the current season (15 micrograms HA for each strain per one 0.5 ml dose) and slight differences were reported in the list of excipients. However, we do not have any information on the manufacturing process and stability of the drug substance that could affect IVE. Finally, we can not exclude that some confounding variables not considered in the data analysis could represent a potential bias.

Our findings are in line with the interim results recently reported from six studies across Europe on IVE estimates among all ages in primary care settings, which ranged between 32% and 43%; higher values were detected against A(H1N1)pdm09 (from 45% to 71%) compared to A(H3N2) (from −39% to 24%) **[14]**. In the United States, the interim estimate of 47% vaccine effectiveness against influenza A(H1N1)pdm09 in all age groups was similar to that observed in European countries **[15]**, whereas higher values were found in Canada (72%) **[16]** and Australia (78%) **[17]**.

In the present season, a co-circulation of A(H1N1)pdm09 and A(H3N2) viruses was reported in Italy, with molecular characteristics similar to influenza A viruses circulating in

Table 3. Influenza vaccine effectiveness, 2018/19 season in Italy.

	Vacc. cases	Unvacc. cases	Vacc. controls	Unvacc. controls	Crude IVE	95% CI		Adj. IVE	95% CI	
Virus type										
A	147	1,027	187	1,162	11.1	−12.1	29.5	25.0	0.4	43.6
A(H1N1)	50	534	187	1,162	41.8	19.2	58.1	44.8	18.8	62.5
A(H3N2)	96	480	187	1,162	−24.3	−62.5	4.9	1.8	−37.8	30.1
Virus type by age groups										
6 months-17 years										
A	46	582	46	574	1.4	−50.8	35.5	22.6	−20.9	50.4
A(H1N1)pdm09	9	274	46	574	59.0	15.1	80.2	65.7	27.5	83.8
A(H3N2)	37	302	46	574	−52.9	−140.9	3.0	−13.4	−84.7	30.4
18–64 years										
A	46	421	67	547	10.8	−32.6	40.0	31.0	−5.3	54.8
A(H1N1)pdm09	21	248	67	547	30.9	−15.4	58.6	37.1	−8.2	63.4
A(H3N2)	25	166	67	547	−23.0	−100.9	24.8	11.4	−50.9	47.9
≥65 years										
A	55	24	74	41	−27.0	−134.3	31.2	1.7	−92.2	49.8
A(H1N1)pdm09	20	12	74	41	7.7	−107.8	59.0	13.1	−105.2	63.2
A(H3N2)	34	12	74	41	−57.0	−235.9	26.6	−16.7	−171.6	49.8
Virus type by vaccine type and brand										
QIV										
A	118	1,027	141	1,162	5.3	−22.6	26.9	22.6	−4.6	42.8
A(H1N1)pdm09	34	534	141	1,162	47.5	22.6	64.4	50.3	23.4	67.7
A(H3N2)	84	480	141	1,162	−44.2	−92.8	−7.9	−7.8	−53.7	24.3
Vaxigrip Tetra (QIV)										
A	69	1,027	97	1,162	19.5	−10.8	41.5	32.7	2.5	53.6
A(H1N1)pdm09	18	534	97	1,162	59.6	32.5	75.8	62.5	34.3	78.6
A(H3N2)	51	480	97	1,162	−27.3	−81.6	10.8	−4.8	−61.0	31.7
Fluarix Tetra (QIV)										
A	49	1,027	44	1,162	−26.0	−90.9	16.8	3.8	−50.7	38.6
A(H1N1)pdm09	16	534	44	1,162	20.9	−41.5	55.8	24.5	−40.6	59.6
A(H3N2)	33	480	44	1,162	−81.6	−188.7	−14.2	−12.9	−91.3	33.4
Fluad (aTIV)										
A	24	1,027	35	1,162	22.4	−31.3	54.2	21.4	−49.5	58.7
A(H1N1)pdm09	13	534	35	1,162	19.2	−54.0	57.6	2.0	−116.8	55.8
A(H3N2)	10	480	35	1,162	30.8	−40.8	66.0	35.2	−50.8	72.1
Vaccine brand by age groups										
6 months-17 years										
QIV	44	582	44	574	1.4	−52.1	36.1	24.3	−20.1	52.3
Vaxigrip Tetra (QIV)	15	582	20	574	26.0	−45.9	62.5	43.5	−14.9	72.3
Fluarix Tetra (QIV)	29	582	24	574	−19.2	−107.2	31.5	7.9	−66.1	49.0
18–64 years										
QIV	42	421	55	547	0.8	−51.2	34.9	28.3	−13.5	54.7
Vaxigrip Tetra (QIV)	27	421	44	547	20.3	−30.9	51.4	40.0	−2.9	65.0
Fluarix Tetra (QIV)	15	421	11	547	−77.2	−289.7	19.5	−11.9	−151.9	50.3
≥65 years										
QIV	32	24	42	41	−30.2	−157.5	34.2	3.1	−105.7	54.3
Vaxigrip Tetra (QIV)	27	24	33	41	−39.8	−186.0	31.7	1.5	−116.9	55.3
Fluarix Tetra (QIV)	5	24	9	41	5.1	−216.3	71.5	8.9	−233.2	75.1
Fluad (aTIV)	23	24	31	41	−26.7	−165.1	39.4	−1.1	−121.9	57.8

Vacc, vaccinated, Unvacc, unvaccinated, Adj, adjusted. QIV, quadrivalent inactivated vaccine; aTIV, adjuvanted trivalent inactivated vaccine. Crude and adjusted IVE were estimated using the univariable and multivariable logistic regression models. Confounding factors used for the adjusted estimates were sex, age, chronic conditions, date of ILI symptoms onset.

other European countries [18]. In particular, although a degree of molecular heterogeneity was observed among circulating A (H1N1)pdm09 viruses, they maintained antigenic similarity to the vaccine strain (A/Michigan/45/2015) as measured by hemagglutination inhibition (HI) assays using post-infection ferret antisera raised against the vaccine virus. However, a lower reactivity of the circulating viruses with the HA amino acid substitution S183P was measured against post-vaccination human antisera, that may have contributed to the low IVE observed in elderly individuals [19]. The molecular and phylogenetic analyses performed on the HA gene of A (H3N2) viruses highlighted that they clustered mainly in the 3C.2a1b subclade (70.6%), with amino acid changes compared to the vaccine strain (A/Singapore/INFIMH-16-0019/2016, 3C.2a1 subclade). In addition, the detection of A(H3N2) viruses belonging to 3C.2a2 subclade, although at low levels of circulation, and the emergence of the antigenically distinct 3C.3a strains may explain the lack of effectiveness of the vaccines in preventing influenza caused by A(H3N2) viruses. Of note, a moderate IVE against A(H3N2) was measured in the elderly immunized with Fluad®, thus providing further evidence of the ability of adjuvanted vaccine formulation to confer cross-protection against heterovariant influenza strains [20,21]. Conversely, the low IVE detected against A(H1N1) pdm09 could be due to a potential bias by the previous infection- and/or vaccination-derived immunity persisting from one season to the next [22,23]. Hence, the integration of virological data seems essential to interpret IVE results, particularly when low IVE against a specific clade is found or when several clades are circulating in the population. This situation highlights the difficulties in accurately and timely anticipating antigenic changes in influenza viruses for the

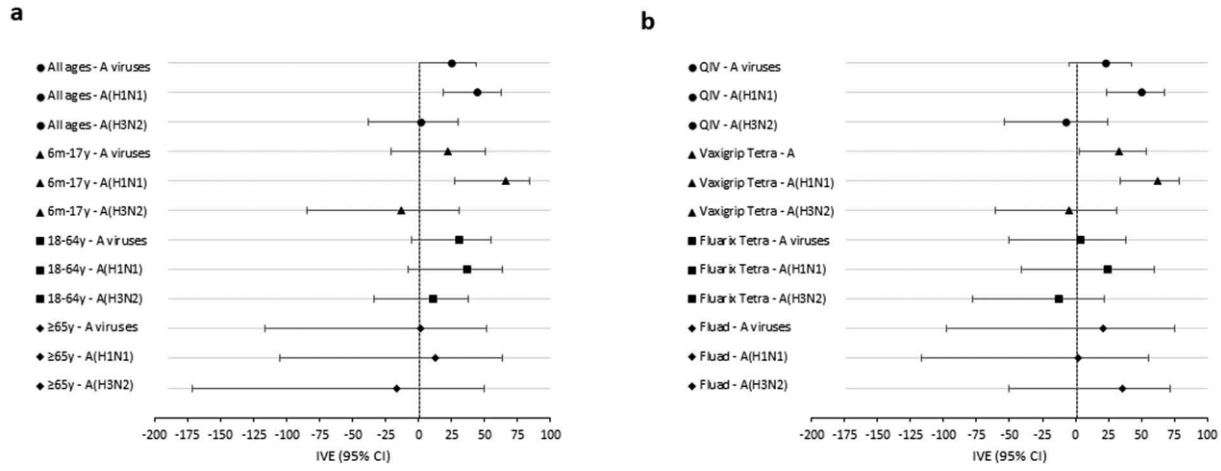

Figure 3. Influenza vaccine effectiveness, 2018/19 season in Italy.

selection and subsequent inclusion of the proper antigenic variants in the vaccine composition.

In interpreting our results, we need to consider some limitations. The low number of subjects in some subgroups and the consequent low statistical power resulted in wide confidence intervals of the IVE estimates, which prevent to draw definitive conclusions. Nevertheless, it is difficult to know the sample size required to obtain robust and precise age-specific IVE estimates by virus type and vaccine brand, as it depends on many unknown factors, including influenza attack rates, vaccination coverage, and distribution of vaccine brands. A test-negative design has notable strengths in minimizing misclassification of disease, using laboratory-confirmed influenza as an outcome measure, and controlling for confounding by healthcare-seeking behavior since study subjects are patients who visit medical institutions due to ILI. However, IVE is supposed to be the same in those who seek care for ILI and in those who do not, assuming that both cases and controls are likely to have the same extent of experience in their exposure to influenza virus [24]. Further, limitations potentially present in all observational studies include residual confounding factors, despite we were able to collect the most important known confounding variables [6]. Negative confounding may occur as high-risk groups are more likely to be vaccinated and therefore reduce IVE, conversely, positive confounding may occur as a result of a 'healthy vaccine effect' leading to an increase of measured IVE [25]. Moreover, vaccine coverage is low in frail elderly patients; consequently, there may be relatively fewer severely ill patients in the exposed group [26,27]. In addition, the increasing number of people immunized against influenza annually has drawn attention on the effect of repeated vaccination, since they could cause either positive or negative interference [22,28].

The most important objective for the next season will be the enhancement of the coverage in the Italian population, in order to increase the sample size and therefore to obtain more precise estimates, particularly by vaccine type and brand. Moreover, another main aspect will be to reinforce the integration between epidemiological and virological data, improving the representative selection of clinical specimens for genetic and antigenic analysis.

5. Conclusions

Although vaccination remains the best tool for the prevention of the influenza illness, in recent years the vaccine was less effective against influenza A(H3N2) viruses [29,30]. Our findings confirm the need for effective interventions against this virus subtype, such as the use of neuraminidase inhibitors to help prevent severe outcomes irrespective of vaccination status [31], and the use of new cell-grown influenza vaccine, which may have an improved match to circulating influenza strains as they avoid egg-adaption issues [32].

Authors' contributions

SB wrote the manuscript, AB coordinated InfluNet, and performed the statistical analysis; SP, ADM, and MF contributed to the virological data collection, and performed genomic sequencing and phylogenetic analyses; OP and PP critically revised the manuscript; MRC coordinated the Italian reference laboratory network for influenza, analyzed the virological data and revised the manuscript. InfluNet Study Group participated to the study. All authors read and approved the manuscript.

Data availability statement

Data supporting the results reported in the article come from the National Influenza Surveillance system database that is available at the Istituto Superiore di Sanità (https://old.iss.it/site/RMI/influnet/pagine/rapportoInflunet.aspx).

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with

the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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