ORIGINAL ARTICLE

Influenza vaccine effectiveness in children: a retrospective study on eight post-pandemic seasons with trivalent inactivated vaccine

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Summary. *Background and aim of the work*: The global burden of disease attributable to seasonal influenza virus in children is difficult to quantify. Children with chronic medical conditions and healthy children may experience severe or fatal complications. Aim of the study was to estimate the influenza vaccine effectiveness (VE) in a cohort of outpatient children. *Methods:* From 2010 to 2018, a Pediatrician of Parma from the InfluNet network of Emilia-Romagna Region, performed nasal/throat swabs on every child with Influenza-like illness at least 14 days from the vaccination with trivalent vaccine. VE estimates against influenza season, virus type and subtype and age group were evaluated using a test-negative design. *Results:* 2,480 swabs were performed. The 57.6% of the analyzed swabs were positive for influenza viruses. Type A (57%) and type B viruses (43%) co-circulated. The 37.1% of type A viruses belonged to subtype A(H3N2), 19.4% to subtype A(H1N1)pdm09. The subtype A(H3N2) was prevalent among children up to 23 months (42.4%) while the type B in the 2-4 (40.7%) and 5-16 year old age groups (49.4%). Overall, 19.9% of the children were vaccinated. The highest prevalence of vaccinated subjects was found in children aged 5-16 (30.5%). The VE against subtype A(H1N1)pdm09 was 63% (95%CI 42.6-76.0), against type B 27.5% (95%CI 7.9-42.9) and against subtype A(H3N2) -14.3% (95%CI - 46.0-10.7). *Conclusions:* Our findings represent a useful contribution to the ongoing debate about the appropriateness of including influenza vaccination for healthy children, 6 months and older, in the updating National Vaccine Prevention Plan (PNPV).

Key words: influenza, children, outpatient, virological surveillance, vaccine effectiveness, test-negative design.

Introduction

The global burden of disease attributable to seasonal influenza virus (SIV) is difficult to quantify, particularly in children younger than 5 years, as pediatric hospitalization rates and SIV-related deaths vary by the predominant circulating strain, and from season to season. For instance, a study conducted by Poheling et al. (1), underlined as among young children, outpatient visits associated with influenza were 10 to 250 times as common as hospitalizations. Moreover, children with chronic medical conditions, as well as healthy children,

may experience severe or fatal complications, with a substantial number of excess hospitalizations, medical visits, antibiotic use and deaths (2).

Nair et al., in a sistematic review (3), estimated that, in 2008, 90 million new cases of influenza, 20 million cases of influenza-associated ALRI (acute, lower respiratory infections) (13% of all cases of paediatric ALRI), and 1 million cases of influenza-associated severe ALRI (7% of cases of all severe paediatric ALRI) occurred worldwide in children younger than 5 years. They estimated there were 28,000–111,500 deaths in children younger than 5 years attributable to

influenza-associated ALRI in 2008, with 99% of these deaths occurring in developing countries.

Lafond et al. (4) in 2016, with a different survey methodology, confirmed the great impact of influenza on pediatric hospitalizations and estimated that influenza was associated with 10% of respiratory hospitalizations in children <18 years of age worldwide, ranging from 5% among children <6 mounths to 16% among children 5–17 years, with 374,000 hospitalizations in children <1 years and 870,000 hospitalizations in children <5 years annually.

According to American Academy of Pediatrics (5), during 2018/2019 influenza season, 51% of deaths attributable to influenza occurred in children who had at least 1 underlying medical condition; therefore, nearly half had unknown underlying medical conditions. Among the children hospitalized with influenza, 45% had no recorded underlying condition, and 55% had at least 1 underlying medical condition (asthma or reactive airway disease (27.1%)).

Nonetheless, children play a central role in the transmission of influenza virus infection to household and other close contacts (6). As shown by Principi et al., (7) the number of medical visits, and the number of missed working or school days, were all significantly greater among the household contacts of influenza positive children than those of children infected by other agents. On the other hand, children have often the highest attack rates in the community during seasonal influenza epidemics (20%-30% in children vs 5%-10% in adults) (8).

Universal seasonal vaccine administration to everyone 6 months and older is the best available strategy to prevent SIV complications (9-10), but studies focusing on estimates of vaccine effectiveness (VE) are still required (11-19). In order to contribute to a better understanding of such topic, we performed a retrospective study on eight post pandemic influenza seasons (2010-2018). More specifically, in order to produce seasonal influenza VE estimates, we established a test-negative (TN) study design in a cohort of outpatient children within the context of integrated virological and epidemiological surveillance, coordinated by the Istituto Superiore di Sanità (ISS) and conducted in Emilia-Romagna (Northern Italy), at the Regional Reference Laboratory of Parma.

Methods

Subjects in study

From 2010/2011 to 2017/2018, a Pediatrician of Parma, from the InfluNet network of Emilia-Romagna Region, with an average number of 1,149 assisted (4% of all pediatric residents of the Province of Parma) performed nasal or throat swabs, on every child who went to his medical clinic with body temperature > 37.5 °C and at least one symptom among those included in the definition of pediatric ILI (Influenza-Like Illness) (20), (i.e. dry or productive cough, pharyngodynia, nasal/cold congestion, conjunctivitis, chills, asthenia, muscle and osteoarticular pain, irritability, crying, loss of appetite) within 4 days from the beginning of the symptoms and at least 14 days from the vaccination. Each biological sample, marked with a code, was accompanied by a data collection card that reported: the date of birth, sex, the date of the beginning of the symptoms, and the vaccination status. The study was approved by the Provincial Ethical Committee (CEP) as an observational study and a written informed consent document was collected for each subject both for the execution of the swab and for the collection of data.

Virological investigation

The "Virocult" diagnostic kit (MWE, England) was used to collect the clinicals samples. Each sample was delivered into refrigerated box to the Laboratory and was analyzed within 24 hours of arrival. Laboratory diagnosis was undertaken by using one-step Real Time retro-transcription PCR assay (rRT-PCR), able to detect circulating influenza A and B viruses and subtypes, according to CDC (Centers for Disease Control and Prevention) and WHO (World Health Organization) protocols (21-22). Viral nucleic acid was extracted from respiratory specimens using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). A rRT-PCR was performed with Quantifast Pathogen+IC Kit, (Qiagen, Hilden, Germany). From 2013/2014 season, genetic lineage of type B (B Yamagata/Victoria lineage) was also determined. All assays were performed using the Rotor Gene 6000 (Corbett).

Statistical analysis

The results were summarized in tables of frequency and the differences in the proportions were compared by the use of Chi square test, with Yates's correction of continuity when appropriate. The distribution of subjects' age was summarized by mean, standard deviation (SD) and median, and tested with Anova.

In relation to the epidemiological trend and according to the viral circulation monitored by the Italian InfluNet network (23), every influenza season was divided into three phases: first one ascending from the 46th week, a peak phase corresponding to the week with the highest number of positive samples more or less 2 weeks, and a downward phase. Children were stratified into three age groups: 0-23 months, 2-4 year of age, and 5-16 year of age.

Under the TN design, subjects who seek medical care for ILI and tested positive for influenza virus infection by RT-PCR are cases, subjects who seek medical care for ILI and tested negative by RT-PCR for influenza virus infection are controls (24-26).

We estimated the VE as 1-OR*100 with the relative confidence intervals of 95%. A logistic regression model was used to calculate the adjusted VE (i.e. outcome variable) for sex, age group and epidemic period (i.e. covariates). In particular, were estimated: the overall influenza VE (8 years) (adjusted for epidemic period, age group and sex); the VE against every influenza season (adjusted for epidemic period, age group and sex); the VE against subtype A(H1N1)pdm09, subtype A(H3N2) and type B (adjusted for epidemic period, age group and sex); the VE against age group (adjusted for epidemic period, and sex).

P-values equal to or less than 0.05 were considered statistically significant. All statistical analyses were performed with SPSS 25.0 (IBM SPSS Inc., Chicago – IL).

Results

During the 8 influenza seasons, a total of 2,480 nasal or throat swabs were performed; the highest number of samples was analyzed in the 2012/2013 season (368, 14.8% of all samples), the lowest in the 2013/2014 season (145, 5.8 % of all samples) (Table 1). Study population had a mean age of 4.7 years (SD 3.5), a median age of 4 years (range: 3 months to 16 years), with a male/

Table 1. Subjects in study by influenza season.

Influenza Season	Subjects (No.) (%)	Sex Male (No.) (%)	Sex Female (No.) (%)	Sex Missing (No.) (%)	Age Ratio M/F	Age Mean (SD)	Age Median	Age group 0-23 mounth (No.) (%)	Age group 2-4 years (No.) (%)	Age group 5-16 years (No.) (%)	Age group Missing (No.) (%)
2010/2011	276 (11.4)	141 (51.1)	134 (48.6)	1 (0.3)	1.05	5.0 (3.5)	5.0	52 (18.9)	84 (30.4)	137 (49.6)	3 (1.1)
2011/2012	364 (14.7)	183 (50.3)	181 (49.7)	0 (0)	1.01	4.0 (3.1)	3.0	84 (23.1)	156 (42.8)	124 (34.1)	0 (0)
2012/2013	368 (14.8)	179 (48.7)	183 (49.7)	6 (1.6)	0.98	4.4 (3.2)	4.0	70 (19.0)	148 (40.2)	150 (40.8)	0 (0)
2013/2014	145 (5.8)	79 (54.5)	63 (43.4)	3 (2.1)	1.25	4.8 (3.8)	3.0	32 (22.1)	52 (35.9)	61 (42.0)	0 (0)
2014/2015	339 (13.6)	176 (51.9)	163 (48.1)	0 (0)	1.08	4.7 (3.5)	4.0	75 (22.2)	115 (33.9)	149 (43.9)	0 (0)
2015/2016	348 (14.0)	183 (52.6)	165 (47.4)	0 (0)	1.11	4.9 (3.5)	4.0	73 (21.0)	106 (30.4)	169 (48.6)	0 (0)
2016/2017	305 (12.2)	178 (58.4)	127 (41.6)	0 (0)	1.40	5.4 (4.0)	4.0	45 (14.8)	122 (40.0)	138 (45.2)	0 (0)
2017/2018	335 (13.5)	217 (64.8)	118 (35.2)	0 (0)	1.84	4.3 (3.5)	3.0	73 (21.8)	144 (43.0)	118 (35.2)	0 (0)
Total	2480 (100)	1336 (53.9)	1134 (45.7)	10 (0.4)	1.18	4.7 (3.5)	4.0	504 (20.3)	927 (37.4)	1046 (42.2)	3 (0.1)

female ratio of 1.18. Overall, 19.9% of the children were vaccinated with inactivated trivalent vaccine (Table 2). The 57.6% of the analyzed swabs were positive for influenza viruses (range 27.6% to 71.2%; Table 2).

During the 8 considered seasons, type A (57%) and type B viruses (43%) co-circulated. The 37.1% of type A viruses belonged to subtype A(H3N2), 19.4% to subtype A(H1N1)pdm09, and the remaining 0.5% was not subtyped. The highest number of samples was collected between the 4th and 6th week of each season in the first 5 epidemic seasons, during the 7th week in the 2015/2016 season, during the 51st week in the 2016/2017 season (3rd week of December 2016) and during the 3rd week of 2018 in the 2017/2018 season (Figure 1).

The season with the highest percentage of the viral isolations was 2012/2013 (71.2%), characterized by the co-circulation of subtype A(H1N1)pdm09 (20.2%) and type B (79.4%), followed by the 2011/2012 season (70.6%) during which subtype A(H3N2) circulated almost exclusively (98.4%). The most evident co-circulation of the 2 subtypes A(H1N1)pdm09 (40.0%) and A(H3N2) (52.8%) was observed in 2014/2015 season
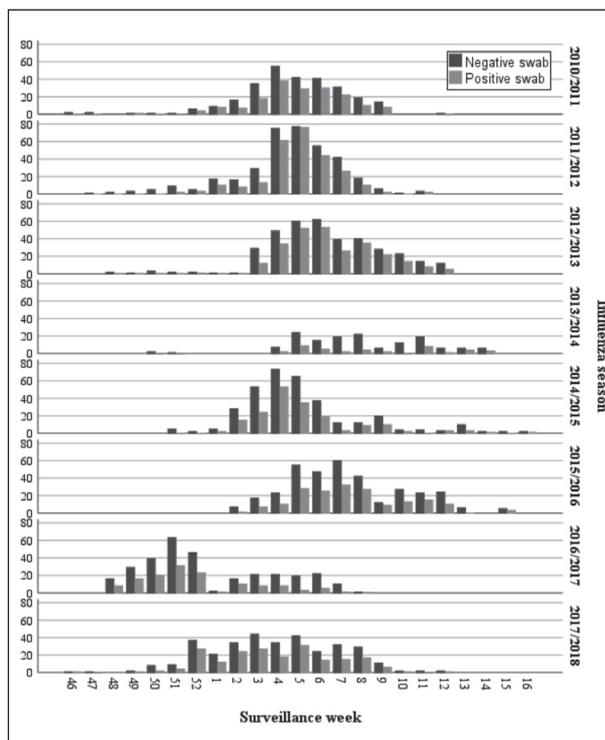
Figure 1. Number of negative and positive swabs by influenza season and surveillance week

Table 2. Influenza virus type/subtype and vaccination status by influenza season.

| Influenza Season | Vaccination status | | Positive samples/ total swabs | | Influenza virus type or subtype | | | | | |
	Vaccinated (No.) (%)	Unvaccinated (No.) (%)	(No.)	(%)	A unsubtyped (No.) (%)	A(H3N2) (No.) (%)	A(H1N1)pdm09 (No.) (%)	Influenza B (No.) (%)	Lineage B/Vic (%)	Lineage B/Yam (%)
2010/2011	101 (36.6)	175 (63.4)	175/276	(63.4)	4 (2.3)	3 (1.7)	55 (31.4)	113 (64.6)	-	-
2011/2012	117 (32.1)	247 (67.9)	257/364	(70.6)	3 (1.2)	253 (98.4)	0 (0)	1 (0.4)	-	-
2012/2013	57 (15.5)	311 (84.5)	262/368	(71.2)	0 (0)	1 (0.4)	53 (20.2)	208 (79.4)	-	-
2013/2014	41 (28.3)	104 (71.7)	40/145	(27.6)	0 (0)	33 (82.5)	6 (15.0)	1 (2.5)	0	100
2014/2015	66 (19.5)	273 (80.5)	180/339	(53.1)	0 (0)	95 (52.8)	72 (40.0)	13 (7.2)	0	100
2015/2016	53 (15.2)	295 (84.8)	180/348	(53.1)	0 (0)	11 (6.1)	7 (3.9)	162 (90.0)	98.8	1.2
2016/2017	28 (9.2)	277 (90.8)	134/305	(43.9)	0 (0)	132 (98.6)	1 (0.7)	1 (0.7)	0	100
2017/2018	31 (9.2)	304 (90.8)	201/335	(60.0)	0 (0)	2 (1.0)	83 (41.3)	116 (57.7)	0.9	99.1
Total	494 (19.9)	1986 (80.1)	1429/2480	(57.6)	7/1429 (0.5)	530/1429 (37.1)	277/1429 (19.4)	615/1429 (43.0)		

(Table 2). The subtype A(H1N1)pdm09 frequently co-circulated with type B, while the circulation of the other strains was substantially residual in 3 out of the 4 seasons in which the subtype A(H3N2) was prevalent.

The prevalence of sampled vaccinated children decreased during the study: 36.6% in the immediate post-pandemic season, 9.2% and 9.2% in the 2016/2017 and 2017/2018 influenza seasons (Table 2). The percentage of influenza-positive samples was significantly different in the three age groups: 43.0% among children up to 23 month of age, 55.3% in 2-4 year old children, and 66.6% in older children (p<0,001) (Table 3). The subtype A(H3N2) was prevalent among the youngest children up to 23 months (42.4% of viruses isolated in this age group), while the type B was prevalent in the 2-4 year-old age group (40.7%) and in the 5-16 year-old age group (49.4%). Overall, in the 8 seasons, the highest prevalence of vaccinated subjects was found in the group of children aged 5-16 (30.5%), without differences between males and females (Table 3).

VE analysis.

Table 4 shows the VE estimates by epidemic season, by viral type or subtype and by age group. Briegly, considering the 8 epidemic seasons, the overall VE was 37.1% (95% CI 22.2 - 49.2). In 5 of the 8 analyzed seasons, the VE exceeded 50%, ranging from 56% (95% CI 21.1 - 75.5) in 2011/2012 season to 68.9% (95% CI 21.9 - 87.6) in the 2016/2017 season. In 3 seasons, i.e. from 2013 to 2016, the VE was moderate. Specifically, in 2014/2015 season, characterized by the co-circulation of the subtypes A(H1N1)pdm09 and A(H3N2), the VE was 38.2% (95% CI -13.5 - 66.3), while in 2015/2016 season, characterized by the predominant circulation of the B virus (90%), specially B/Victoria lineage (98.8%), VE showed negative values. In the 2013/2014 season, the low number of positive samples made VE estimates unreliable. Overall, the VE against subtype A(H1N1)pdm09 was 63% (95% CI 42.6 - 76.0), against type B 27.5% (95% CI 7.9 - 42.9) and against subtype A(H3N2) -14.3% (95% CI - 46.0 - 10.7). Among the age groups, the VE estimates against younger children under 23 months, were comparable with those aged 5-16, although VE estimates were statistically significant only in this class: 43.1% (95% CI -90.2 – 83.4), and 42.9% (95% CI 23.4

Table 3. Vaccination status and influenza virus type/subtype by age group.

| Age group | Vaccination status | | Positive samples/total swabs | Influenza virus type or subtype | | | | |
	Vaccinated (No.) (%)	Unvaccinated (No.) (%)	(No.) (%)	A unsubtyped (No.) (%)	A(H3N2) (No.) (%)	A(H1N1)pdm09 (No.) (%)	Influenza B (No.) (%)
0-23 mounths	13 (2.6)	491 (97.4)	217/504 (43.0)	1 (0.5)	92 (42.4)	63 (29.0)	61 (28.1)
2-4 years	160 (17.3)	767 (82.7)	513/927 (55.3)	4 (0.8)	182 (35.5)	118 (23.0)	209 (40.7)
5-16 years	319 (30.5)	727 (69.5)	697/1046 (66.6)	2 (0.3)	256 (36.7)	95 (13.6)	344 (49.4)
Missing	2 (66.7)	1 (33.3)	2/3 (66.7)	0 (0)	0 (0)	1 (50.0)	1 (50.0)
Total	494	1986	1429/2480 (57.6)	7/1429 (0.5)	530/1429 (37.1)	277/1429 (19.4)	615/1429 (43.0)

Table 4. Adjusted vaccine effectiveness estimates (VE) against influenza seasons, influenza type and subtype, and age group.

	P	Adjusted VE %	Adjusted 95% CI	
			Lower	Upper
Overall	< 0.001	37.1	22.2	49.2
VE= (1- ORadj) x 100		OR adjusted for epidemic period, age group and sex		
Against Influenza season				
2010/2011	< 0.05	62.0	30.7	79.2
2011/2012	< 0.05	56.0	21.1	75.5
2012/2013	< 0.05	60.5	24.8	79.2
2013/2014	n.s.	2.7	- 137.2	60.1
2014/2015	n.s.	38.2	- 13.5	66.3
2015/2016	n.s.	- 9.9	- 112.7	43.1
2016/2017	< 0.05	68.9	21.9	87.6
2017/2018	< 0.05	60.5	13.6	81.9
VE= (1- ORadj) x 100		OR adjusted for epidemic period, age group and sex		
Against Influenza type and subtype				
A(H1N1)pdm09	< 0.001	63.0	42.6	76.0
A(H3N2)	n.s.	- 14.3	- 46.0	10.7
B	< 0.05	27.5	7.9	42.9
VE= (1- ORadj) x 100		OR adjusted for epidemic period, age group and sex		
Against age group				
0 - 23 mounths	n.s.	43.1	- 90.2	83.4
2 – 4 years	n.s.	27.5	- 3.8	48.2
5 – 16 years	< 0.001	42.9	23.4	56.7
VE= (1- ORadj) x 100		OR adjusted for epidemic period and sex		

– 56.7) respectively. The lowest value was found in the intermediate age class (2-4 year-old) in which the VE was 27.5% (95% CI -3.8 – 48.9).

Conclusions

The eight epidemic seasons considered in our study were characterized by the frequent co-circulation of influenza A and B viruses. Although the percentage of vaccinated children decreased during the course of the study, the coverage rate of our children remained widely above the regional average coverage (1.85%) (27). This permitted to obtain robust VE estimates. Overall, the effectiveness of the vaccination was good but, as already observed in other studies and in different age groups (28), it was high against the subtype A(H1N1)pdm09 (63%) and substantially lower against the subtype A(H3N2) and towards the type B.

In particular, in 2014/2015 season, characterized by the co-circulation of the two subtypes A, the low VE value (38.2%; 95% CI -13.5 - 66.3) was supported by an important mismatch of the circulating A(H3N2) strain, genetically and antigenically different from the vaccine strain; in the following season (2015/2016), locally characterized by the intense circulation of type B virus, the presence in the trivalent formulation vaccine of the lineage B/Yamagata, different from the circulating lineage B/Victoria, may have determined the low VE value (27.5% ; 95% CI 7.9 - 42.9).

Interestingly in both seasons the relative prevalence of locally circulating strains was different from that observed at National level: in fact, in Italy in 2014/2015 the subtype A(H3N2) represented 41% of the viruses against 52.8% of our study, and in 2015/2016 the type B in Italy represented 57% of the viruses against 90% of our study (29-30).

This could account the lower protection of the tri-

valent vaccine observed in our paediatric population compared to that observed in other studies (31) and underlines the relevance of local surveillance systems that can provide more appropriate data and information in specific population groups (32-35).

Our study has some limitations: although the TN design controls for health care seeking behaviour bias, the VE estimates may not be generalizable to entire population (36). We adjusted the VE estimates for age, sex and epidemic season period. However, for accurate VE estimation, it will be necessary to consider, in the future, also a severity score, based on the clinical symptomatology of the disease for each patient; furthermore, the low number of vaccinated children under two year old, did not allow to calculate reliable VE estimates, exactly in this age group, where the greatest questions remain in terms of cost-effectiveness of vaccination, a common theme in many diseases (37), and one of the major drivers in public health decisions. However, the scenario could change quickly: the recent indication to propose vaccination in all pregnant women could determine, in addition to protecting themselves, an increase in protection in newborns as for other vaccine preventable diseases (11,38). Furthermore, in Emilia-Romagna Region, as well as in Italy, the trivalent vaccine has been recently replaced by the quadrivalent vaccine, which should show greater efficacy against B strains, in particular in children. On the other hand, influenza vaccination in Italy in children from 6 months of age, is still voluntary and provided only with payment.

In conclusion, our findings represent a useful contribution to the ongoing debate about the appropriateness of including influenza vaccination for healthy children, 6 months and older, in the updating National Vaccine Prevention Plan (PNPV).

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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