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Influenza vaccine effectiveness estimates in Croatia in 2010–2011: a season with predominant circulation of A(H1N1)pdm09 influenza virus

S. KUREČIĆ FILIPOVIĆ[1]*, I. GJENERO-MARGAN[2], E. KISSLING[3], B. KAIĆ[1] AND A. CVITKOVIĆ[4]

[1] *Epidemiology Department, Croatian National Institute of Public Health, Zagreb, Croatia*
[2] *University of Applied Health Studies, Zagreb, Croatia*
[3] *EpiConcept, Paris, France*
[4] *Public Health Institute, Brodsko-Posavska County, Slavonski Brod, Croatia*

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SUMMARY

This is a retrospective study using the test-negative case-control method to estimate seasonal 2010–2011 influenza vaccine effectiveness (VE) in Croatia. Of patients consulting a physician for influenza-like illness (ILI) and for whom a swab was taken, we compared RT–PCR influenza-positive and RT–PCR influenza-negative patients. We used a structured questionnaire and physicians' records to obtain information on vaccination status and potential confounders. We conducted a complete case analysis using logistic regression to measure adjusted VE overall, against A(H1N1)pdm09 and in age groups. Out of 785 interviewed patients, 495 eligible patients were included in the study, after applying exclusion criteria [217 cases, of which 92·6% were A(H1N1)pdm09 positive, 278 controls]. Crude VE was 31·9% [95% confidence interval (CI) −40·9 to 67·1] and adjusted VE was 20·7% (95% CI −71·4 to 63·3), with higher VE in youngest and oldest age groups. Results from this first VE study in Croatia suggest a low to moderate VE for the 2010–2011 season. Studies year on year are needed with a greater sample size to provide more precise estimates, and also by age group and risk groups for vaccination.

Key words: Influenza, influenza vaccines, vaccination (immunization).

INTRODUCTION

In Croatia the influenza season usually occurs between Christmas and Easter and influenza vaccination starts in October or November. Influenza is a mandatory notifiable disease according to Croatian legislation which includes laboratory-confirmed cases and reports based only on clinical investigation [1, 2]. Reporting of individual influenza cases is mandatory throughout the entire year, but during the influenza season the reporting is aggregated weekly by age groups. The Croatian National Institute of Public Health (CNIPH) acts as a national WHO influenza centre and is responsible for both epidemiological and virological surveillance of influenza.

According to the CNIPH Epidemiology Service data, from 2002 to 2009, about 11–14% of the Croatian population received vaccination against influenza each year [3]. In the last decade only subunit and split trivalent influenza vaccines were used in Croatia.

In the 2010–2011 season the influenza vaccination campaign started on 12 November 2010. The influenza virus composition of the 2010–2011 seasonal trivalent

* Author for correspondence: Dr S. Kurečić Filipović, Epidemiology Department, Croatian National Institute of Public Health, Rockefellerova 7, 10000 Zagreb, Croatia.
(Email: sanja.kurecicfilipovic@hzjz.hr)

influenza vaccine was as follows: A/California/07/2009 (H1 subtype), A/Perth/16/2009 (H3 subtype) and B/Brisbane 60/2008 viruses, as recommended by WHO in February 2010 [4]. The influenza A(H1) strain was the same as that used in the monovalent 2009–2010 pandemic vaccine, which showed good effectiveness in preventing A(H1N1)pdm09 influenza in 2009–2010 [5]. The CNIPH Epidemiology Service recommended influenza vaccination primarly for the following groups: all persons aged $\geqslant 65$ years, individuals aged $\geqslant 6$ months with the following chronic medical conditions: chronic heart and respiratory diseases, chronic diseases of metabolism (including diabetes mellitus), chronic kidney and liver diseases, haemoglobinopathy and immunosuppression. In addition, the influenza vaccine was recommended to people with severe obesity, children and adolescents (age 6 months to 18 years) on long-term therapy with medications containing acetylsalicylic acid, healthcare professionals, elderly residents of care homes and institutions providing care for the chronically ill (regardless of age, including children) and employees of the same institutions. Household members of persons recommended for influenza vaccination but who have contraindications for vaccination or persons providing medical care to such persons are also target groups for vaccination.

The Croatian Health Insurance Fund provided free influenza vaccination for these target groups. Croatian citizens can also receive vaccination outside the funded programme. Around 470 000 influenza vaccine doses were administered. There have been no influenza vaccine effectiveness (VE) studies using epidemiological methods in Croatia so far. The aim of this case-control study was to estimate the VE of the 2010–2011 trivalent seasonal influenza vaccine in the prevention of laboratory-confirmed influenza in Croatia.

METHODS

In the 2010–2011 influenza season virological surveillance was performed, as usual, by the CNIPH Virology Department, acting as a WHO national influenza laboratory. Samples from influenza like illness (ILI) patients were collected from 14 December 2010 to 1 June 2011 and tested for influenza. For reporting purposes the 2008 European Union ILI case definition was used: sudden onset of symptoms and at least one of the following four systematic symptoms (fever or feverishness, malaise, headache, myalgia) and at least one of the following three respiratory symptoms (cough, sore throat, shortness of breath) [6].

The selection of ILI patients from whom the samples were taken was made at the physician's discretion. However there was a general recommendation to take samples from patients with a severe clinical indication. Therefore most samples were taken in the hospital setting.

Nucleic acids were isolated from respiratory specimens and placed in viral transport medium (Hanks). RNA was extracted using an automated system QIAxtractor (Qiagen, USA).

Real-time reverse transcriptase–polymerase chain reaction (RT–PCR) was applied to the detection of viral RNA using a single-tube RT–PCR kit according to the manufacturer's instructions (Invitrogen SuperScript™ III Platinum® One-Step Quantitative kit, USA). Amplification and detection were performed with a 7500 Real Time PCR System machine (Applied Biosystems, USA).

Using random number tables we randomly selected 1000 patients from the ILI patients with a RT–PCR result and interviewed them by phone using a structured questionnaire. The study was approved by CNIPH Ethics Committee and all participants provided oral consent.

The following data were collected: date of birth, gender, 2010–2011 seasonal influenza vaccination status (including place of vaccination and name of the vaccinator), time elapsed between vaccination and disease onset, time elapsed between disease onset and specimen collection, information on admission to hospital, vaccination with seasonal and pandemic influenza vaccine in the previous 2009–2010 season, comorbidities, pregnancy status, smoking status (never, stopped more than a year ago, active smoker), if the patient has been vaccinated against influenza regularly for at least 2 years, and the reason for vaccination. In cases where the patient reported they had received an influenza vaccination, the vaccine provider was contacted and full information on vaccination was obtained (exact date of vaccination, name of the vaccine).

We classified ILI patients as a case if they tested positive for influenza virus by RT–PCR. We classified those testing negative as controls.

Patients were considered unvaccinated if there was <14 days between the date of vaccination and disease onset. Participants who had been interviewed were excluded from the VE analysis if they had contraindications for influenza vaccination, if there was an

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interval >7 days between disease onset and specimen collection, or this information was missing, and if the date of specimen collection was after week 14, 2011, the week after which influenza circulation was negligible. We excluded from the VE estimate patients with an interval unknown or >7 days between disease onset and specimen collection due to the fact that influenza virus shedding after day 7 is rare [7].

VE was estimated as $(1 - \mathrm{OR}) \times 100\%$, where the odds ratio (OR) is the ratio of odds of cases being vaccinated to the odds of controls being vaccinated. We performed a complete case analysis where records with missing data were dropped. Multivariable logistic regression was used to calculate ORs and 95% confidence intervals (CIs). VE was adjusted for age, presence of a chronic condition (including pregnancy), sex, month of specimen collection and smoking status (current *vs.* non-current smoker).

Stata v. 12·0 (StataCorp LP, USA) was used for all analyses. Fisher's exact test was used to compare proportions, with $P < 0{\cdot}05$ considered statistically significant.

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

RESULTS

In the 2010–2011 influenza season the CNIPH Epidemiology Service received 55281 ILI notifications via the routine communicable diseases surveillance system.

The incidence of ILI notifications, as reported via weekly aggregated reports, was highest in the youngest age group (0–19 years); 2% of the Croatian population in that age group had clinical influenza. ILI incidence in the 20–64 years age group was 1170/100 000 population, meaning that 1% of the Croatian population in that age group had clinical influenza. The lowest incidence was in persons aged ⩾65 years (0·25% of that age group had clinical influenza) (Table 1).

Between 14 December 2010 and 1 June 2011 the CNIPH Virology Department tested samples from 3660 ILI patients for influenza. In 1692 ILI patients a direct immunofluorescence assay (DFA) was the method used for determining influenza. The RT–PCR method was used to determine influenza in 1968 ILI patients.

Table 1. *Influenza-like illness (ILI) notifications by age group, Croatia, season 2010–2011*

Age group (years)	No. of ILI notifications	Incidence per 100000 population
0–19	22237	2111
20–64	331254	1170
⩾65	1790	251

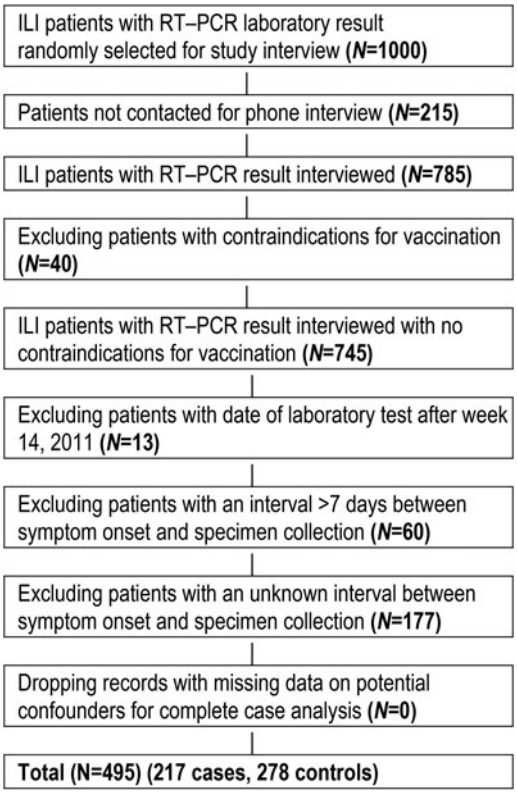

Fig. 1. Flowchart of data exclusion for vaccine effectiveness analysis, influenza season 2010–2011, Croatia. RT–PCR, Reverse transcriptase–polymerase chain reaction; ILI, influenza-like illness.

Samples were sent from all 21 Croatian counties. Out of the 1968 patients, 872 (44%) were positive for influenza. Out of 1000 randomly selected ILI patients with a RT–PCR laboratory result, 785 patients were contacted by phone and interviewed. Of the 785 patients interviewed, 40 had contraindications for vaccination and were excluded from the VE analysis, as well as 250 patients whose interval between symptom onset and specimen collection was unknown or reported to be >7 days, and patients with laboratory results after week 14, 2011 (Fig. 1).

A total of 495 ILI patients were included in the study. Of these, 217 (44%) were cases and 278 (56%)

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Table 2. *Characteristics of cases and controls, vaccine effectiveness study, Croatia, season 2010–2011 (N = 495)*

		Cases (*N* = 217)		Controls (*N* = 278)		
		N	(%)	*N*	(%)	*P* value
Age (years)	Median age	27·0		27·5		0·856
	Missing	0		0		
Age group (years)	0–17	79	(36·4)	113	(40·6)	0·029
	18–64	118	(54·4)	122	(43·9)	
	⩾65	20	(9·2)	43	(15·5)	
	Missing	0		0		
Sex	Male	105	(48·4)	164	(59·0)	0·023
	Female	112	(51·6)	114	(41·0)	
	Missing	0		0		
Seasonal 2010–2011 influenza vaccination	Yes	12	(5·5)	22	(7·9)	0·371
	No	205	(94·5)	256	(92·1)	
	Missing	0		0		
Influenza virus type	Control	–	–	278	(100·0)	
	A(H1N1)pdm09	201	(92·6)	0	–	
	B	16	(7·4)	0	–	
Any reported chronic condition	Yes	87	(40·1)	131	(47·1)	0·122
	No	130	(59·9)	147	(52·9)	
	Missing	0		0		
Delay between symptom onset and specimen collection	<4 days	167	(77·3)	211	(75·9)	0·749
	⩾4 days	49	(22·7)	67	(24·1)	
	Missing	1*		0		
Early and late phase of the influenza season	Before week 5, 2011	82	(37·8)	125	(45·0)	0·119
	After week 5, 2011	135	(62·2)	153	(55·0)	
	Missing	0		0		
Smoker	Active	25	(11·5)	29	(10·4)	0·906
	Former	20	(9·2)	28	(10·1)	
	Never	172	(79·3)	221	(79·5)	
	Missing	0		0		
Month of specimen collection	December	11	(61·3)	18	(57·9)	.
	January	133	(18·9)	161	(14·7)	
	February	41		41		
	March	30		53		
	April	2		5		
	Missing	0		0		
Institution type sending the specimens for influenza testing	Public health institute	7	(3·2)	3	(1·1)	0·157
	University/clinical hospital	151	(69·6)	190	(68·3)	
	General hospital	58	(26·7)	85	(30·6)	
	General Practitioner	1	(0·5)	0	(0·0)	
	Missing	0		0		

* For this record it was known that the delay between symptom onset and specimen collection was 3–6 days, but the exact number of days was unknown.

were controls. The median age of cases and controls was 27 years [interquartile range (IQR) 7–48 years] and 27·5 years (IQR 3–53 years), respectively (Table 2). Controls comprised 41·0% female, and cases 51·6% female ($P = 0·023$). Of controls 47·1% had presence of a chronic condition compared to 40·1% of cases ($P = 0·122$). With respect to delay between symptom onset and specimen collection, 24·1% of controls had a delay of 4–7 days as did 22·7% of cases ($P = 0·749$). Of controls, 55·0% of specimens were collected after week 5, 2011 compared to 62·2% of cases. Specimens were sent by medical specialists working in the hospitals for 98·9% of controls and 96·3% of cases.

Influenza virus A(H1N1)pdm09 was predominantly circulating in the 2010–2011 season (Fig. 2).

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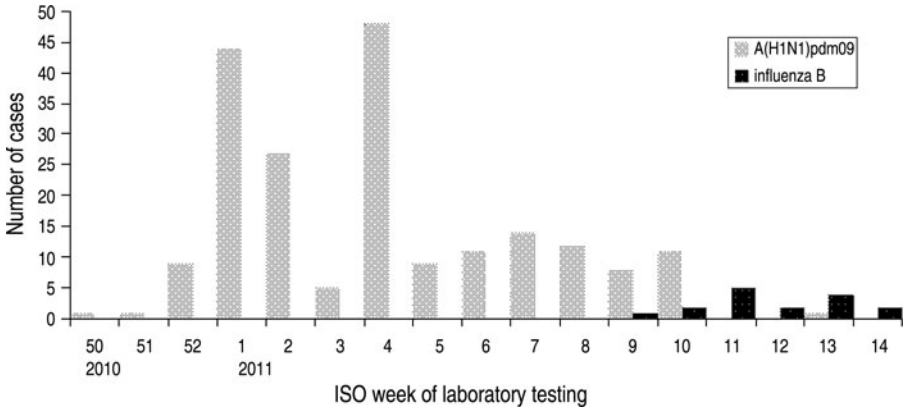

Fig. 2. Influenza cases by virus subtype by International Organization for Standardization (ISO) week of laboratory testing, Croatia, 2010–2011 ($N = 495$).

Table 3. *Number and vaccination status of cases and controls by age group, vaccine effectiveness study, Croatia, season 2010–2011 (N = 495)*

	Age group (years)			
	0–17	18–64	⩾65	Total
Cases				
No.	79	118	20	217
Vaccinated (%)	1 (1·3)	8 (6·8)	3 (15·0)	12 (5·5)
Controls				
No.	113	122	43	278
Vaccinated (%)	4 (3·5)	7 (5·7)	11 (25·6)	22 (7·9)

In total, 201 (92·6%) cases had A(H1N1)pdm09 influenza virus and 16 (7·4%) cases had influenza B virus confirmed by RT–PCR.

Of the 495 patients eligible for VE analysis, 34 (6·9%) were reported as vaccinated (12 cases, 22 controls) ($P = 0·371$).

Vaccination coverage varied by age group and status of cases and controls; of those aged ⩾65 years, 22·2% were vaccinated (15·0% of cases, 25·6% of controls) (Table 3). In the 0–17 years age group, vaccination coverage was low (2·6%), with only one vaccinated case.

The crude VE against any influenza was 31·9% (95% CI −40·9 to 67·1) and against influenza A(H1N1)pdm09 it was 33·4 (−40·6 to 68·5) (Table 4). The fully adjusted VE estimates were 20·7% (95% CI −71·4 to 63·3) and 17·3% (95% CI −84·1 to 62·9) against any influenza and influenza A(H1N1)pdm09, respectively.

Crude VE estimates against any influenza by age group were 65·1% (95% CI -215·6 to 96·2), −19·5% (95% CI −240·6 to 58·1) and 48·7% (95% CI −109·4 to 87·4) for the 0–17, 18–64 and ⩾65 years age groups, respectively (Table 5). Crude VE estimates against influenza A(H1N1)pdm09 were 63·2% (95% CI −236·1 to 96·0), −27·6% (95% CI −264·2 to 55·3) and 61·2% (95% CI −97·3 to 92·4) for the 0–17, 18–64 and ⩾65 years age groups, respectively.

DISCUSSION

This test-negative case-control study suggests overall low VE in 2010–2011 for the seasonal influenza vaccine used in Croatia. Crude VE against any influenza was estimated at 31·9% (95% CI -40·9 to 67·1) for all ages ($N = 495$) and adjusted VE as 20·7% (95% CI −71·4 to 63·3). This is different from other 2010–2011 influenza VE estimates in Europe for all ages where results suggest moderate protection from 2010–2011 trivalent vaccine against medically attended ILI laboratory-confirmed influenza across Europe [8, 9].

Age-specific crude VE estimates against any influenza and against influenza A(H1N1)pdm09 were higher in the 0–17 and ⩾65 years age groups than for those aged 18–64 years. The age-specific crude VE estimates against any influenza for the 0–17 and ⩾65 years age groups were 65·1% and 48·7%, respectively, and against influenza A(H1N1)pdm09 were 63·2% and 61·2%, respectively. The crude VE estimate for the 18–64 years age group was −19·5% against any influenza and −27·6% against influenza A(H1N1)pdm09. However, confidence intervals were wide for all age-specific estimates.

While the precision around the estimates was low, the estimates for the 0–17 and ⩾65 years age groups were comparable to estimates from a European multicentre case-control study [8]. In the European

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Table 4. *Crude and adjusted vaccine effectiveness of seasonal influenza vaccine against any influenza and against influenza A(H1N1)pdm09, Croatia, season 2010–2011*

	Any influenza ($N = 495$)		Influenza A(H1N1)pdm09 ($N = 476$)	
	VE	95% CI	VE	95% CI
Main analysis				
Crude	31·9	−40·9–67·1	33·4	−40·6–68·5
Adjusted for				
Age group	23·0	−64·3–63·9	21·4	−71·6–63·9
Chronic condition	24·3	−59·2–64·0	23·8	−63·7–64·5
Month of specimen collection*	30·6	−44·2–66·6	30·0	−50·0–67·3
Sex	31·7	−41·9–67·1	33·7	−40·7–68·7
Smoking	31·7	−41·3–67·0	33·4	−40·7–68·5
Age group + chronic condition	20·1	−71·1–62·7	18·0	−79·4–62·5
Age group + chronic condition + month of specimen collection*	18·9	−74·6–62·3	14·9	−88·7–61·6
Age group + chronic condition + month of specimen collection + sex*	20·8	−70·9–63·3	17·4	−83·9–62·9
Age group + chronic condition + month of specimen collection + sex + smoking*	20·7	−71·4–63·3	17·3	−84·1–62·9

VE, Vaccine effectiveness; CI, confidence interval,
* April dropped for A(H1N1)pdm09 analysis (two observations).

Table 5. *Crude vaccine effectiveness of seasonal vaccine against influenza by age group, Croatia, season 2010–2011*

	Any influenza			Influenza A(H1N1)pdm09		
Age group	*N*	Crude VE (%)	95% CI	*N*	Crude VE (%)	95% CI
0–17	192	65·1	−215·6 to 96·2	183	63·2	−236·1 to 96·0
18–64	240	−19·5	−240·6 to 58·1	233	−27·6	−264·2 to 55·3
⩾65	63	48·7	−109·4 to 87·4	60	61·2	−97·3 to 92·4

CI, Confidence interval.

multicentre study, estimates in the adult age range were also lower compared to the younger and older age groups.

In 2010–2011 influenza season there was a good match between the vaccine and circulating A and B influenza virus strains as reported by the Community Network of Reference Laboratories (CNRL) for Human Influenza in Europe [10]. Antigenic analysis of A(H1N1) viruses by HI (turkey RBCs) reported by CNRL in a summary report in March 2011 included test viruses from Croatia [11].

A very wide interval of the 95% CIs of the adjusted VE estimates indicates that the results of this retrospective study should be interpreted with caution and that a larger sample size is needed for more conclusive results.The precision of VE estimates in Croatia is influenced by the sample size and vaccination coverage. In addition to only a limited number of ILI patients with RT–PCR results available in the country, we interviewed only a sample of those patients. The questionnaire was administered retrospectively and it proved difficult to reach patients for the phone inteview after the influenza season ended. However, due to the questionnaire design, we were able to collect information on a variety of potential confounders and apart from the data on interval between disease onset and specimen collection, the data completeness was about 100%.

Vaccine coverage of trivalent seasonal influenza vaccine in controls in our study was 8% which is below the average Croatian influenza vaccine coverage of about 11% for this season.

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The age distribution of the study population is different than the age distribution of the Croatian population. The proportion of older people is higher in the Croatian population than it is seen in this study population, which may explain the lower vaccine coverage.

The 2010–2011 VE study in Croatia is subject to the usual biases of an observational study. It uses the test-negative design, which is a design that still needs to be thoroughly validated. These biases have been described by other authors in detail [5, 7, 12]. In addition, the selection of patients to swab by clinicians was done at the discretion of the clinician, which may have introduced a bias. However, clinicians were blinded to the case status of patients, so this bias may be limited.

VE estimates are affected by the specificity of outcome [13]. Laboratory confirmation is the preffered method in VE studies as it offers the most specific outcome [14]. The method of obtaining the specimen should be standardized as it may influence the specificity of the outcome [7]. It may be that clinicians in Croatia did not take the swabs in a systematic manner and bias was introduced.

We have also applied the study questionnaire retrospectively, which may have caused recall bias in patients. Patients had difficulties in remembering how many days had elapsed between onset of symptoms and specimen collection (out of 672 patients in the study population and within the study period, 177 could not remember how many days had elapsed). We have excluded from the VE analysis patients with missing information on period between disease onset and specimen collection. Additionally persons who responded to this question might be different in terms of case and vaccination status from patients who could not answer this question.

We did not collect information on antiviral prescriptions and therefore could not assess the influence of antivirals (neuraminidase inhibitors) on virus shedding.

Age group was the strongest confounder in the study. VE estimates decreased by around 9% absolutely when adjusting for age. In this study, A (H1N1)pdm09 influenza virus mostly affected younger age groups, vaccination coverage was also different for the different age groups, which caused this confounding.

Still, this rather low influenza VE estimate in Croatia could also be explained by several factors that made the 2010–2011 influenza season unique. During the 2009–2010 pandemic, 0·4% of the Croatian population were vaccinated against influenza with the monovalent pandemic vaccine, which is far below the numbers reported for the majority of European countries [15]. In our study, out of 495 participants only six received 2009 pandemic vaccine. Some studies estimated a higher VE in patients who had received both 2010–2011 seasonal trivalent and 2009 monovalent pandemic vaccines [9]. The second dose acting as a booster has been reported by other authors [16–18]. As the majority of persons in our study were not vaccinated with the pandemic vaccine, a potential booster effect may be missing. However, in the 2010–2011 season reported VE was low to moderate in many countries [19, 20].

Some 2010–2011 mid-season VE studies have mentioned the possible role of antigenic drift and different study populations as possible explanations of lower 2010–2011 influenza VE in comparison to pandemic VE [21, 22].

After considering all the limitations of this retrospective test-negative case-control study we believe this study has several strengths: this is the first influenza VE study in Croatia to explore the possibility of using epidemiological methodology. We believe that such an initiative will further increase the importance of understanding the epidemiology of influenza and influenza surveillance. In an influenza season with a low number of ILI notifications the power of small studies to detect VE may be compromised [23]. Due to variability in ILI rates, influenza type/subtypes and vaccine match from season to season it is important to estimate VE in several seasons or even annually. Having country-specific VE estimates is important as each country has unique characteristics: vaccination recommendations and vaccines used might be significantly different between countries and the vaccines administered are supplied by different manufacturers. On the other hand, to obtain influenza type/subtype-specific VE estimates by age group and risk group with reasonable precision, we realize the importance of having the opportunities to increase the sample size. Therefore we also support the initiative of networks that can perform a pooled analysis on data from several countries. A further strength of our study is the very high data completeness obtained in potentially confounding variables.

Despite low to moderate 2010–2011 influenza VE estimates, influenza vaccine should be considered as the most effective measure for prevention of influenza and its complications, especially for the most vulnerable population groups for whom there could be fatal consequences.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Croatian Parliament.** Act on the protection of the population against communicable diseases. *Narodne novine*, No. 79/07 (http://narodne-novine.nn.hr/clanci/sluzbeni/2007_07_79_2486.html). Accessed 31 May 2012.
2. **Ministry of Health.** List of communicable diseases the control and prevention of which is of interest to Croatia. *Narodne novine*, No. 79/09 (http://narodne-novine.nn.hr/clanci/sluzbeni/2009_07_79_1939.html). Accessed 31 May 2012.
3. **Kaic B.** Influenza vaccination. *Medicus* 2011; **20**: 101–108.
4. **World Health Organization.** Recommended viruses for influenza vaccines for use in the 2010–2011 northern hemisphere influenza season. (http://www.who.int/influenza/vaccines/virus/recommendations/201002_Recommendation.pdf.). Accessed 15 April 2013.
5. **Valenciano M, *et al.*** Estimates of pandemic influenza vaccine effectiveness in Europe, 2009–2010: results of Influenza Monitoring Vaccine Effectiveness in Europe (I-MOVE) multicentre case-control study. *PLoS Medicine*. 2011; **8**: e1000388.
6. **European Commission.** Decision No 2119/98/EC of the European Parliament and of the Council of 24 September 1998 setting up a network for the epidemiological surveillance and control of communicable diseases in the Community (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:159:0046:0090:EN:PDF.). Accessed 15 April 2013.
7. **Valenciano M, *et al.*** Study designs for timely estimation of influenza vaccine effectiveness using European sentinel practitioner networks. *Vaccine* 2010; **28**: 7381–7388.
8. **Kissling E, *et al.*** I-MOVE multi-centre case control study 2010–11: overall and stratified estimates of influenza vaccine effectiveness in Europe. *PLoS ONE* 2011; **6**: e27622.
9. **Jiménez-Jorge S, *et al.*** Effectiveness of the 2010–11 seasonal trivalent influenza vaccine in Spain: cycEVA study. *Vaccine* 2012; **30**: 3595–3602
10. **European Center for Disease Prevention and Control (ECDC).** Influenza virus characterisation, Summary Europe, July 2011 (http://ecdc.europa.eu/en/publications/Publications/influenza-virus-characterisation-CNRL-dec-2012.pdf). Accessed 31 May 2012.
11. **European Center for Disease Prevention and Control (ECDC).** Influenza virus characterisation, Summary Europe, March 2011 (http://ecdc.europa.eu/en/publications/Publications/influenza-virus-characterisation-CNRL-dec-2012.pdf). Accessed 31 May 2012.
12. **Hak E, *et al.*** Confounding by indication in non-experimental evaluation of vaccine effectiveness: the example of prevention of influenza complications. *Journal of Epidemiology and Community Health* 2002; **56**: 951–955.
13. **Orenstein WA, *et al.*** Field evaluation of vaccine efficacy. *Bulletin of the World Health Organization* 1985; **63**: 1055–1068.
14. **Orenstein EW, *et al.*** Methodologic issues regarding the use of three observational study designs to assess influenza vaccine effectiveness. *International Journal of Epidemiology* 2007; **36**: 623–631.
15. **Mereckiene J, *et al.*** Influenza A(H1N1)pdm09 vaccination policies and coverage in Europe. *Eurosurveillance* 2012; **17**: pii = 20064.
16. **Gilca V, *et al.*** Antibody persistence and response to 2010–2011 trivalent influenza vaccine one year after a single dose of 2009 AS03-adjuvanted pandemic H1N1 vaccine in children. *Vaccine* 2011; **30**: 35–41.
17. **Pebody R, *et al.*** Effectiveness of seasonal 2010/11 and pandemic influenza A(H1N1)2009 vaccines in preventing influenza infection in the United Kingdom: mid-season analysis 2010/11. *Eurosurveillance* 2011; **16**: pii = 19791.
18. **Castilla J, *et al.*** Effectiveness of trivalent seasonal and monovalent influenza A(H1N1)2009 vaccines in population with major chronic conditions of Navarre, Spain: 2010/11 mid-season analysis. *Eurosurveillance* 2011; **16**: pii = 19799.
19. **Puig-Barbera J.** 2010–2011 influenza seasonal vaccine, preliminary mid-season effectiveness estimates: reason for concern, confounding or are we following the right track? *Eurosurveillance* 2011; **16**: pii = 19821.
20. **Steens A, *et al.*** Influenza vaccine effectiveness, 2010/11. *Eurosurveillance* 2011; **16**: pii = 19843.
21. **Kissling E, Valenciano M, I-MOVE case-control studies team**. Early estimates of seasonal influenza vaccine effectiveness in Europe, 2010/11: I-MOVE, a multicentre case-control study. *Eurosurveillance* 2011; **16**: pii = 19818.
22. **Savulescu C, *et al.*** Effectiveness of the 2010/11 seasonal trivalent influenza vaccine in Spain: preliminary results of a case-control study. *Eurosurveillance* 2011; **16**: pii = 19820.
23. **Fiore AE, *et al.*** Inactivated influenza vaccines. In: Plotkin SA, Orenstein SA, Offit PA, eds. *Vaccine*, 6th edn. Philadelphia: Elsevier, 2013, pp. 257–293.