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# Waning protection of influenza vaccine against mild laboratory confirmed influenza A(H3N2) and B in Spain, season 2014–15

Alin Gherasim[a,*], Francisco Pozo[b], Salvador de Mateo[a,c], Inma Aspiritxaga Gamarra[d], Manuel García-Cenoz[c,e], Tomas Vega[f], Eva Martínez[g], Jaume Giménez[h], Daniel Castrillejo[i], Amparo Larrauri[a,c], cycEVA team and the VEVA Working Group[1]

[a] National Centre of Epidemiology, Institute of Health Carlos III, Spain
[b] National Centre for Microbiology, National Influenza Reference Laboratory, WHO-National Influenza Centre, Institute of Health Carlos III, Spain
[c] CIBER Epidemiología y Salud Pública (CIBERESP), Institute of Health Carlos III, Spain
[d] Subdirección de Salud Pública y Adicciones de Bizkaia, Pais Vasco, Spain
[e] Instituto de Salud Pública, Navarra Institute for Health Research (IdiSNA), Pamplona, Spain
[f] Dirección General de Salud Pública, Consejería de Sanidad de Castilla y León, Valladolid, Spain
[g] Servicio de Epidemiología y Prevención Sanitaria, Dirección General de Salud Pública y Consumo de La Rioja, La Rioja, Spain
[h] Servicio de Epidemiología, Dirección General de Salut Pública, Mallorca, Baleares, Spain
[i] Servicio de Epidemiología. DGSC, Consejería de Bienestar Social y Sanidad, Ciudad Autónoma de Melilla, Spain

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ABSTRACT

*Introduction:* The 2014/15 influenza season in Spain was dominated by the circulation of drifted A(H3N2) and co-circulation of B viruses. We present the final estimates of influenza vaccine effectiveness (IVE) against confirmed influenza A(H3N2) and B its evolution along the season and with time since vaccination.
*Methods:* We used data collected on influenza like illness patients (ILI), systematically swabbed for the presence of influenza viruses within the Spanish Influenza Sentinel Surveillance System (SISS) and a restricted observational study (cycEVA). We used a test negative case–control design to compare influenza confirmed cases with negative controls. We estimated the IVE through a logistic regression model adjusting for potential confounders. The evolution of IVE was studied in early and late stages of the epidemic, and in different time intervals between receiving influenza vaccination and the onset of symptoms.
*Results:* At the end of the season we have found low and moderate IVE point estimates against influenza A(H3N2) and B, respectively, in all ages and target groups for vaccination. An IVE decreased from an early value of 37% to a late of −76% against influenza A(H3N2), and similarly, 84% vs −4% against Influenza B. When the onset of symptoms occurred more than three months after vaccination, the decrease of IVE was slower and milder against influenza B than against influenza A(H3N2). No significant change in the percentage of circulating drifted influenza A(H3N2) strains belonging to the 3c.2a and 3c.3a clades could be identified through the season.
*Conclusions:* In a season dominated by drifted A(H3N2) circulating virus, the vaccine offered little or no protection against A(H3N2) infection but had a moderate protective effect against influenza B. Efforts should be put in developing influenza vaccines that maintain their protective capabilities throughout the season and could stimulate a potentially broad immune response against diverse influenza strains.


* Corresponding author at: National Centre of Epidemiology, Institute of Health Carlos III, c/Monforte de Lemos no. 5, 28029 Madrid, Spain. Tel.: +34 918222624; fax: +34 913877815.
*E-mail addresses:* amgherasim@externos.isciii.es (A. Gherasim), pacopozo@isciii.es (F. Pozo), smateo@isciii.es (S. de Mateo), enfer4bi-san@euskadi.eus (I. Aspiritxaga Gamarra), mgcenoz@cfnavarra.es (M. García-Cenoz), VegAloTo@jcyl.es (T. Vega), emochoa@larioja.org (E. Martínez), jgimenez@dgsanita.caib.es (J. Giménez), dcastr01@melilla.es (D. Castrillejo), alarrauri@isciii.es (A. Larrauri).
[1] For members of these teams, please refer to Supplementary data.

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## 1. Introduction

Influenza vaccination is considered the most important intervention towards preventing complications by influenza not only in high-risk groups but also in general population [1,2]. The recommendation for trivalent influenza vaccine for the Northern Hemisphere in the 2014–15 season, included the A/California/7/2009 (H1N1)pdm09, the A/Texas/50/2012 (H3N2) and the B/Massachusetts/02/2012 (Yamagata lineage) strains [3]. The vaccine was offered in Spain to all individuals above 64 years and to those belonging to clinical or professional risk groups [4].

Since the 2008–09 season the (cycEVA) observational case–control study has been monitoring the influenza vaccine effectiveness (IVE) in Spain within the framework of the Influenza Monitoring of Vaccine Effectiveness (I-MOVE) European network. cycEVA has been functioning within the Spanish Influenza Sentinel Surveillance System (SISS), a well-established system of 17 sentinel influenza networks in 17 out of 19 Spanish autonomous regions [5], and has been supplying timely and reliable IVE estimates both during as well as at the end of the season [6–9]. IVE estimates obtained with cycEVA and the entire surveillance system were largely similar [10].

The 2014–15 season in Spain was characterized by the predominant A(H3N2) influenza virus, the majority of which were mismatched with the 2014–15 northern hemisphere A(H3N2) vaccine strain [11].

We present in this paper the final estimates of IVE 2014–15 in Spain obtained using the SISS and the cycEVA study. Moreover, we studied the evolution of the IVE along the season using two strategies. Firstly we compared the IVE estimates in the early and late phase of the epidemic, using both SISS and cycEVA data. Secondly, we studied the effect of time since vaccination (TSV) on the vaccine protection within cycEVA study. Based on the genetic characterization of a representation of detected strains, we tried to provide additional knowledge on the relationship between IVE and circulating strains pattern.

## 2. Methods

### 2.1. The study population

We considered as study population all patients with influenza-like illness (ILI) symptoms attending the sentinel physicians (SPs) including generalist doctors and paediatricians integrated in the SISS (788 SPs) or in the six sentinel networks participating in the 2014–15 cycEVA study (174 SPs). In five out of six cycEVA regions all of SPs participate in both cycEVA and SISS studies, whereas in the sixth only 77% of the SPs belonging to SISS are participating in cycEVA. The components and the requirements for the SISS and cycEVA functionality were previously described [6,10,12]. Overall, SPs within SISS systematically swabbed ILI patients and collected demographic, clinical, virological, vaccination, chronic conditions, obesity and pregnancy status data. Several variables are routinely collected for the cycEVA study: number of hospitalizations for chronic condition, SPs visits in the last year, previous influenza vaccination, smoking habit and influenza vaccination date.

During the season, in the SISS the influenza vaccination status was ascertained through a dichotomous variable (yes/no). However, at the end of the influenza epidemic we obtained retrospectively the vaccination date for the immunized patients attending 594 SPs belonging to 14 SISS sentinel networks. This information allowed to check if the patient was correctly immunized, with the administration of the vaccine 14 days or more before the onset of symptoms. Information on vaccination date was obtained for the 93.4% of the patients notified as having received the Influenza vaccine.

### 2.2. The study design and data analysis

We conducted a test negative case–control study between 8 December 2014 and 19 April 2015 (weeks 50/2014–16/2015). Cases were ILI patients with a Polymerase Chain Reaction (PCR) swab positive for influenza taken less than eight days after onset of ILI symptoms. ILI patients testing negative for all influenza strains were considered controls.

Baseline characteristics of cases and controls were compared using Chi-squared test. We calculated the IVE as (1-OR for vaccination) $\times$ 100 and we used logistic regression models to obtain IVE estimates with 95% confidence intervals (95%CI), adjusted by potential confounders collected both by SISS and cycEVA study.

IVE was estimated by virus type/subtype in all ages and in target groups for vaccination. Additionally we studied the IVE within specific age-groups (0–14; 15–64; >64 years), using SISS data.

We performed two sensitivity analyses: (1) Restricting to patients swabbed 4 days or less after symptoms onset; (2) Using SISS data with vaccination date in order to evaluate the potential differences in IVE estimates when considering all patients as correctly vaccinated, and afterwards only patients vaccinated 14 days or more before symptoms onset.

We evaluated the IVE against A(H3N2) and B along the season with SISS and cycEVA data dividing the epidemic season in two phases: an early stage including the epidemic peak (weeks 50/2014–5/2015) and a late stage (weeks 6–16/2015).

Using cycEVA data, IVE was estimated through modelling the TSV as a categorical variable defining three TSV intervals according to the tertiles of the number of days elapsed between vaccination and onset of symptoms: t1 = from the lowest value until the first tertile, t2: between second and third tertile and t3: between the third tertile until the maximum value. We estimated the IVE adjusting for all the variables collected in cycEVA, for each TSV interval and using the non-vaccinated as reference group. We applied a logistic regression model in order to evaluate the differences between the IVE in the first TSV interval (t1), used as reference, and the IVE in the t2 and t3 [13]. All data analysis was conducted using STATA version 13.

### 2.3. Laboratory methods

Genetic characterization by sequencing the HA1 fragment of the viral hemagglutinin gene was carried out at the WHO National Influenza Centre (Madrid) in a subset of influenza positive samples and/or virus isolates. Phylogenetic analysis of sequences was carried out in order to characterize the specific genetic group of circulating influenza A and B clades. To detect any change in the clades circulating pattern, we analyzed the evolution in time of the overall proportion of discordant strains using the chi-squared for trend.

## 3. Results

The 2014–15 influenza season peaked in week 5/2015 both at national level and within the regional networks integrated in cycEVA study. The season was dominated by influenza A (61% of the confirmed cases in SISS) with 96% of A(H3N2) among the subtyped A viruses. An increasing influenza B virus circulation was identified towards the end of the epidemic.

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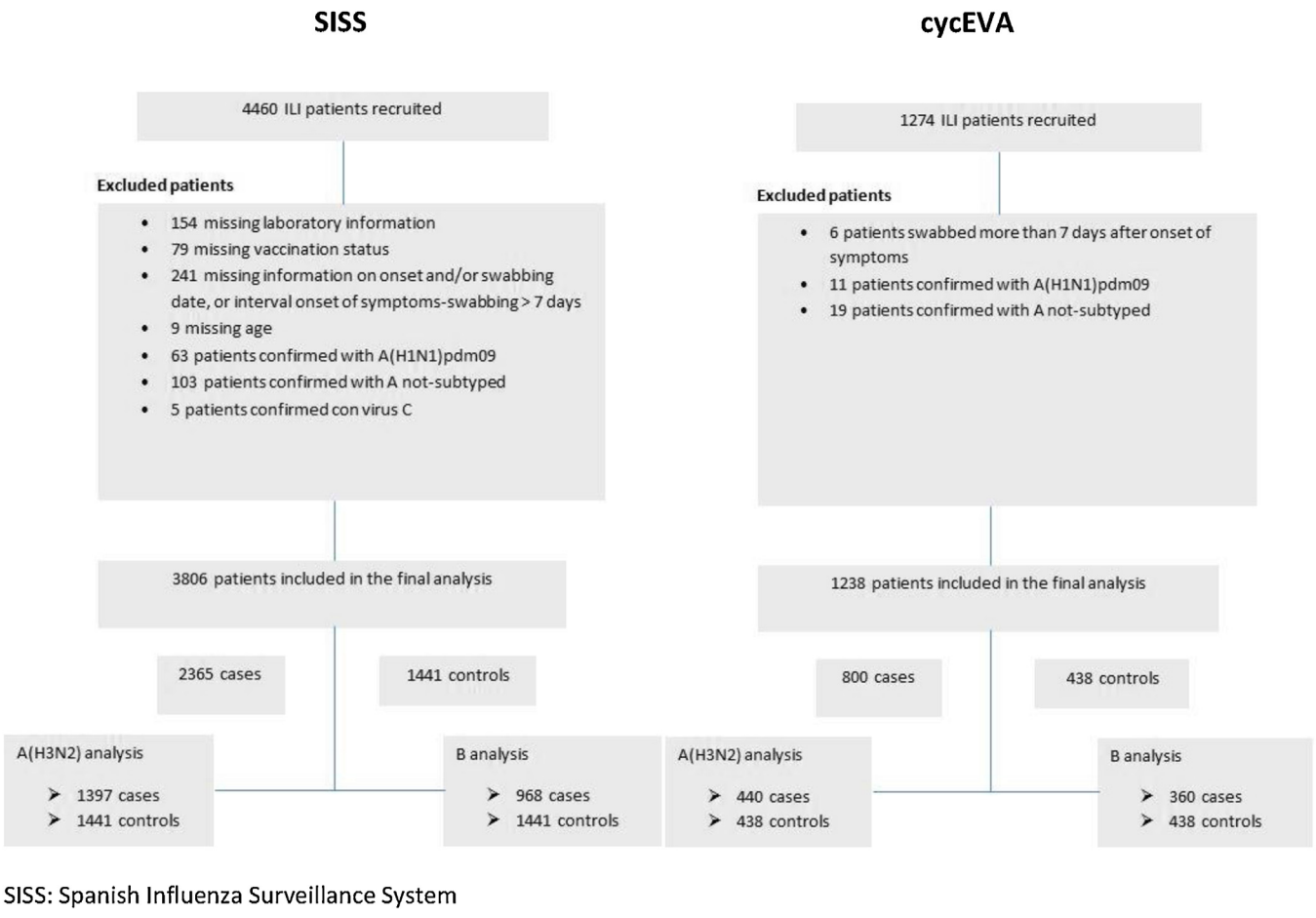


**Fig. 1.** Exclusion criteria, SISS and cycEVA study, influenza season 2014–15 (weeks 50/2014–16/2015), Spain.

### 3.1. SISS study vaccine effectiveness

After applying the exclusion criteria we included in the SISS analysis 1441 negative controls, 2365 confirmed cases (1397 A(H3N2) and 968 B). Within the cycEVA study a total of 438 controls and 800 confirmed cases (440 A(H3N2) and 360 B) were included (Fig. 1). In the SISS, controls were older than A(H3N2) cases (31 vs. 21 years) but younger than B cases (31 vs. 37 years). Individuals in the target group for vaccination were distributed in similar proportions in controls, A(H3N2) and B cases (19.6%, 18.7% and 19.9% respectively). While a similar proportion of controls and A(H3N2) cases were vaccinated (11.9% vs. 10.8%), significantly more controls were vaccinated compared to B cases (11.9% vs. 8.1%).

Within the SISS, the adjusted IVE against A(H3N2) was 16% (95%CI: −11 to 36) and 13% (95%CI: −30; 42) in all ages and target groups for vaccination, respectively (Table 2). The adjusted IVE estimates against influenza A(H3N2) were 17%, 22% and 1% within 0–14, 15–64 and >64 years age-groups, respectively (Table 2). Analysis of sensitivity including those sentinel networks with available vaccination date showed adjusted IVE against A(H3N2) of 6% (95%CI: −30; 33) if considering all vaccinated patients, and 0.5% (95%CI: −40; 29) if considering only those vaccinated 14 or more days before the symptoms onset. Slightly higher IVE point estimates against A(H3N2) were obtained in these analysis for target groups for vaccination (10% and 6%, respectively) (Table 2).

The adjusted IVE against B was 34% (95%CI: 8–52) and 32% (95%CI: −6; 56) in all ages and target groups for vaccination, respectively; by age group comparable adjusted IVE estimates were obtained (31–38%). Analysis of sensitivity in all ages showed an adjusted IVE against B of 27% (95%CI: −7; 51), when considering all vaccinated patients, and 28% (95%CI: −9; 52) when considering only those vaccinated at least 14 days before the symptoms onset. Slightly higher IVE point estimates against B were obtained in these analysis groups for target groups for vaccination (32% and 36%, respectively). Restricting the analysis to those swabbed less than four days since onset of symptoms, we obtained an IVE of 13% (95%CI: −16; 35) and 33% (95%CI: 5; 53) against A(H3N2) and B respectively in all ages.

### 3.2. cycEVA study vaccine effectiveness

Within cycEVA study, controls were also significantly older than A(H3N2) cases (35 vs. 28 years) but were similar with B cases (35 vs. 39 years). Similarly to SISS, there were no differences in the distribution of the individuals in target group for vaccination between controls and A(H3N2) and B cases (22.6%, 23% and 21.7% respectively). Significantly more controls were vaccinated compared to B cases in all ages (10.5% vs. 6.4%), but also in the target groups for vaccination (39.4% vs. 23.1%) (Table 1).

We obtained adjusted IVE against A(H3N2) of 2% (95%CI: −65; 42) and 20% (95%CI: −53; 58) in all ages and target groups for vaccination, respectively (Table 2). Adjusted IVE against B was 48% (95%CI: 4; 71) and 59% (95%CI: 14; 80) in all ages and target groups for vaccination, respectively (Table 2). The analysis restricted to those swabbed less than 4 days yielded in all ages an IVE of −3% (95%CI: −80; 41) and 46% (95%CI: −3; 72) against A(H3N2) and B respectively.

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**Table 1**
Characteristics of influenza A(H3N2) and B cases and negative controls, SISS and cycEVA study, Spain, week 50/2014–16/2015.

| | Controls[a] *n*/*N* (%) | A(H3N2) cases *n*/*N* (%) | B cases *n*/*N* (%) |
|---|---|---|---|
| **SISS study** | | | |
| Age (years) | | | |
| 0–14 | 516/1441 (35.8) | 599/1397 (42.9)* | 285/968 (29.4)* |
| 15–64 | 823/1441 (57.1) | 693/1397 (49.7)* | 618/968 (63.8)* |
| ≥65 | 102/1441 (7.1) | 105/1397 (7.5)* | 65/968 (6.7)* |
| Median age (ICR)[b] | 31 (9–48) | 21 (9–43)* | 37 (12–51)* |
| Male | 703/1438 (48.9) | 684/1393 (49.1) | 479/967 (49.5) |
| Interval symptoms onset-swabbing ≤4 days | 1383/1441 (96) | 1356/1397 (97.1) | 921/968 (95.1) |
| Chronic condition | 178/1417 (12.6) | 182/1375 (13.2) | 113/941 (12.0) |
| Obesity | 12/1411 (0.9) | 12/1368 (0.9) | 19/938 (2)* |
| Pregnancy | 4/1428 (0.3) | 1/1378 (0.1) | 5/947 (0.5) |
| Target groups[c] | 275/1407 (19.6) | 256/1370 (18.7) | 185/929 (19.9) |
| **Vaccination status** | | | |
| All ages | 172/1441 (11.9) | 151/1397 (10.8) | 78/968 (8.1)* |
| Age (years) | | | |
| 0–14 | 37/516 (7.2) | 39/599 (6.5) | 15/285 (5.3) |
| 15–64 | 81/823 (9.8) | 51/693 (7.4) | 35/618 (5.7)* |
| ≥65 | 54/102 (52.9) | 61/105 (58.1) | 28/65 (43.1) |
| Target groups[c] | 108/275 (39.3) | 102/256 (39.8) | 57/185 (30.8) |
| **cycEVA study** | | | |
| Age (years) | | | |
| 0–14 | 126/438 (28.7) | 162/440 (36.8)* | 103/360 (28.6) |
| 15–64 | 280/438 (64.0) | 234/440 (53.2)* | 232/360 (64.3) |
| ≥65 | 32/438 (7.3) | 44/440 (10.0)* | 25/360 (6.9) |
| Median age (ICR) | 35 (13–50) | 28 (6–65)* | 39 (12–74) |
| Male | 227/438 (51.8) | 208/440 (47.3) | 175/360 (48.6) |
| Interval symptoms onset-swabbing ≤4 days | 427/438 (97.5) | 436/440 (99.1) | 342/360 (95.0) |
| Chronic condition | 73/438 (16.7) | 75/440 (17.1) | 51/360 (14.2) |
| Obesity | 7/438 (1.6) | 2/440 (0.5) | 4/360 (1.1) |
| Pregnancy | 1/438 (0.2) | 0/440 (0.0) | 1/360 (0.3) |
| Severity[d] | 4/438 (0.9) | 1/440 (0.2) | 4/360 (1.1) |
| SP consultations[e] | 337/438 (76.9) | 365/440 (83.1)* | 281/360 (78.1) |
| Smoking | 62/438 (14.2) | 43/439 (9.8)* | 51/360 (14.2) |
| Previous influenza vaccination (2013/14) | 38/438 (8.7) | 47/440 (10.7) | 24/360 (6.7) |
| Target groups[c] | 99/438 (22.6) | 101/440 (23.0) | 78/360 (21.7) |
| Vaccination status | | | |
| All ages | 46/438 (10.5) | 50/440 (11.4) | 23/360 (6.4)* |
| Age (years) | | | |
| 0–14 | 4/126 (3.2) | 6/162 (3.7) | 6/103 (5.8) |
| 15–64 | 27/280 (9.6) | 17/234 (7.3) | 9/232 (3.9)* |
| ≥65 | 15/32 (46.9) | 27/44 (61.4) | 8/25 (32.0) |
| Target groups[c] | 39/99 (39.4) | 41/101 (41.0) | 18/78 (23.1)* |
| Previous influenza vaccination (2013/14) | 34/38 (89.5) | 43/47 (91.5) | 19/24 (79.2) |

SISS: Spanish influenza sentinel surveillance system; ICR: inter centile range; SP: sentinel physician; BMI: body mass index.
[a] Comparison A(H3N2) or B vs. controls (Chi-square test).
[b] Non-parametric test of the median.
[c] Patients with at least one of the following conditions: Older than 64 years (older than 59 in 7/17 SISS sentinel networks), obesity (BMI ≥40 kg/m$^2$), pregnancy, chronic disease.
[d] Patients with at least one hospitalization due to chronic condition in the last year.
[e] Patients with at least one GP consultation form any reason in the last year.
* $p < 0.05$.

### 3.3. Vaccine effectiveness in time and with time since vaccination

When we studied the evolution of IVE along the season the adjusted IVE against A(H3N2) decreased from 40% (95%CI: 11; 60) in the early phase to −24% (95%CI: −80; 17) in the late phase of the epidemic within SISS. Similar pattern was obtained within cycEVA study, the IVE decreasing from an early 37% (95%CI: −32; 70) to a late −76% (95%CI: −273; 76) (Table 3).

Analysis of IVE against B showed a decrease from 48% (95%CI: 11; 70) to 30% (95%CI: −6; 53) from early to late phase of the epidemic within SISS. However, in the cycEVA study, a higher difference in adjusted IVE estimates among the two stages of the influenza epidemic was obtained (84% vs. −4%) (Table 3).

To study the IVE evolution with TSV, we generated using cycEVA data, three time intervals t1: 46–88, t2: 89–106 and t3: 107–155 days respectively between influenza vaccination and onset of confirmed influenza A(H3N2); similar intervals were obtained for influenza B. The IVE point estimate against A(H3N2) declined from 46% (95%CI: −23; 76) to −12% (95%CI: −145; 49) and −72% (95%CI: −307; 27), when onset of symptoms occurred up to 88, 106 and 155 days after the vaccination, respectively. The IVE estimates against B, declined from 77% (95%CI: 28; 93) to 62% (95%CI: 0.4; 86) and −25% (95%CI: −218; 51) if vaccinated up to 88, 108 and 172 days before symptoms' onset, respectively (Fig. 2).

The IVE in the third TSV interval (t3) significantly declined in comparison to the first TSV interval (t1) both against A(H3N2) ($p = 0.032$) and against influenza B ($p = 0.019$).

### 3.4. Laboratory results

Through sequencing the HA1 fragment of the viral hemagglutinin gene, the WHO National Influenza Centre genetically

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**Table 2**
Influenza vaccine effectiveness (IVE) against confirmed influenza A(H3N2) and B, SISS and cycEVA study, Spain, weeks 50/2014–16/2015.

| Virus type/subtype | Source | Population group | Vaccinated/total cases (%) | Vaccinated/total controls (%) | Crude IVE % (95%CI) | Adjusted IVE % (95%CI) |
|---|---|---|---|---|---|---|
| A(H3N2) | SISS: 788 SPs | All ages[a] | 151/1397 (10.8) | 172/1441 (11.9) | 11 (−13; 29) | 16 (−11; 36) |
| | | 0–14 years[b] | 39/599 (6.5) | 37/516 (7.2) | 10 (−43; 43) | 17 (−44; 52) |
| | | 15–64 years[b] | 51/693 (7.4) | 81/823 (9.8) | 27 (−5; 50) | 22 (−15; 48) |
| | | >64 years[b] | 61/105 (51.8) | 54/102 (52.9) | −23 (−113; 29) | 1 (−90; 49) |
| | | Target groups[c,d] | 102/256 (39.8) | 108/275 (39.3) | −2 (−45; 27) | 13 (−30; 42) |
| | SISS: 594 SPs (Vaccination yes/no[e]) | All ages | 100/819 (12.2) | 122/983 (12.4) | 2 (−30, 26) | 6 (−30; 33) |
| | | Target groups | 63/150 (42) | 76/181 (42) | 0 (−55; 35) | 10 (−51; 46) |
| | SISS: 594 SPs (Vaccination date[f]) | All ages | 94/813 (11.6) | 113/974 (11.6) | 0.4 (−33; 26) | 0.5 (−40; 29) |
| | | Target groups | 60/147 (40.8) | 74/179 (41.3) | 2 (−52; 37) | 6 (−58; 44) |
| | cycEVA: 174 SPs | All ages | 50/440 (11.4) | 46/438 (10.5) | −9 (−67; 28) | 2 (−65; 42) |
| | | Target groups[g] | 41/101 (40.6) | 39/99 (39.4) | −5 (85; 40) | 20 (−53; 58) |
| B | SISS: 788 SPs | All ages | 78/968 (8.1) | 172/1441 (11.9) | 35 (14; 51) | 34 (9; 53) |
| | | 0–14 years | 15/285 (5.3) | 37/516 (7.2) | 28 (−33; 61) | 38 (−36; 72) |
| | | 15–64 years | 35/618 (7.4) | 81/823 (9.8) | 45 (17; 64) | 38 (3; 60) |
| | | >64 years | 28/65 (43.1) | 54/102 (52.9) | 33 (−26; 64) | 31 (−40; 66) |
| | | Target groups | 57/185 (30.8) | 108/275 (39.3) | 31 (−1; 54) | 32 (−6; 56) |
| | SISS: 594 SPs (Vaccination yes/no[e]) | All ages | 58/588 (9.9) | 122/983 (12.4) | 23 (−8; 45) | 27 (−7; 51) |
| | | Target groups | 41/113 (36.3) | 76/181 (41.2) | 21 (−28; 52) | 32 (−19; 61) |
| | SISS: 594 SPs (Vaccination date[f]) | All ages | 54/584 (9.3) | 113/974 (11.6) | 22 (−9; 45) | 28 (−9; 52) |
| | | Target groups | 38/110 (34.6) | 74/119 (41.3) | 25 (−23; 54) | 36 (−13; 64) |
| | cycEVA: 174 SPs | All ages | 23/360 (6.4) | 46/438 (10.5) | 42 (2; 65) | 48 (4; 71) |
| | | Target groups | 18/78 (23.0) | 39/99 (39.4) | 54 (10; 76) | 59 (14; 80) |

SISS: Spanish influenza sentinel surveillance system; 95%CI: 95% confidence intervals; BMI: body mass index; SP: sentinel physician.
Pre-epidemic and post-epidemic period – weekly ILI incidence below baseline threshold for the 2014/15 season.
Epidemic period: ILI incidence above baseline threshold for the 2014/15 season.
[a] IVE adjusted for sex, age-group (0–14; 15–64; >64 years), chronic condition, sentinel network, time period (pre-epidemic – week 50/2014–01/2015; epidemic (week 02–11/2015); post-epidemic week (11–16/2015).
[b] IVE adjusted for sex, chronic condition, sentinel network, time period (pre-epidemic – week 50/2014–01/2015; epidemic (week 02–11/2015); post-epidemic week (11–16/2015).
[c] Patients with at least one of the following: age >64 years (>59 in 7/17 SISS sentinel networks), obesity (BMI ≥40 kg/m$^2$), pregnancy, chronic condition.
[d] IVE adjusted for sex, age-group (0–14; 15–64; >64 years), sentinel network, time period (pre-epidemic – week 50/2014–01/2015; epidemic (week 02–11/2015); post-epidemic week (11–16/2015).
[e] All patients considered vaccinated if vaccination recorded as "yes".
[f] Only patients vaccinated more than 14 days before onset of symptoms were considered as vaccinated; patients vaccinated less than 14 days excluded from the analysis.
[g] Patients with at least one of the following: age >64 years (>59 in 3/6 cycEVA sentinel networks), obesity (BMI ≥40 kg/m$^2$), pregnancy, chronic condition.

**Table 3**
Influenza vaccine effectiveness (IVE) in early versus late epidemic stage by type/subtype of influenza viruses, SISS and cycEVA study, Spain, week 50/2014–16/2015.

| Data source | Time period | Controls | A(H3N2) | | B | |
|---|---|---|---|---|---|---|
| | | Vaccinated/total (%) | Vaccinated/total cases (%) | Adjusted IVE% (95%CI) | Vaccinated/total cases (%) | Adjusted IVE % (95%CI) |
| SISS | Entire season[a] | 172/1441 (11.9) | 151/1397 (10.8) | 16 (−11; 36) | 78/968 (8.1) | 34 (9; 53) |
| | Early phase[b] | 85/685 (12.4) | 69/847 (8.2) | 40 (11; 60) | 23/341 (6.7) | 48 (11; 70) |
| | Late phase[b] | 87/756 (11.5) | 82/550 (14.9) | −24 (−80; 17) | 55/627 (8.8) | 30 (−6; 53) |
| cycEVA | Entire season[a] | 46/438 (10.5) | 50/440 (11.4) | 2 (−65; 42) | 23/360 (6.4) | 48 (4; 71) |
| | Early phase[b] | 27/202 (13.4) | 20/227 (8.8) | 37 (−32; 70) | 3/120 (2.5) | 84 (41; 95) |
| | Late phase[b] | 19/236 (8.0) | 30/213 (14.1) | −70 (−261; 19) | 20/240 (8.3) | −4 (−133; 53) |

SISS: Spanish influenza sentinel surveillance system.
[a] IVE adjusted for sex, age-group (0–14; 15–64; >64 years), chronic condition, sentinel network, and time period (pre-epidemic – week 50/2014–01/2015; epidemic (week 02–11/2015); post-epidemic week (11–16/2015).
[b] IVE adjusted for sex, age-group (0–14; 15–64; >64 years), chronic condition, sentinel network.

characterized at national level 299 A(H3N2) virus: 32 belonged to the clade 3c.3 represented by A/Samara/73/2013, 70 belonged to the 3c.3b clade, represented by A/Newcastle/22/2014, 148 belonged to the clade 3c.2a represented by A/HongKong/5738/2014 and 49 to the clade 3c.3a represented by A/Switzerland/9715293/2013. All the viruses included in the clades represented by strains A/Switzerland/9715293/2013 and A/Hong Kong/5738/2014 were antigenically different from the vaccine strain A/Texas/50/2012 A(H3N2).

Regarding the influenza B, 100 virus were characterized, of which 98 were similar to B/Phuket/3073/2013-like (Yamagata lineage), antigenically similar to the vaccine strain B/Massachusetts/2/2012; the other two virus belonged to the Victoria lineage represented by B/Brisbane/60/2008 strain.

The proportion of circulating A(H3N2) virus genetically characterized as discordant with the A/Texas/50/2012 vaccine strain increased from 59% between weeks 40/2014 and 1/2015 to 63% at the epidemic's peak and 73% after the epidemic's peak (weeks

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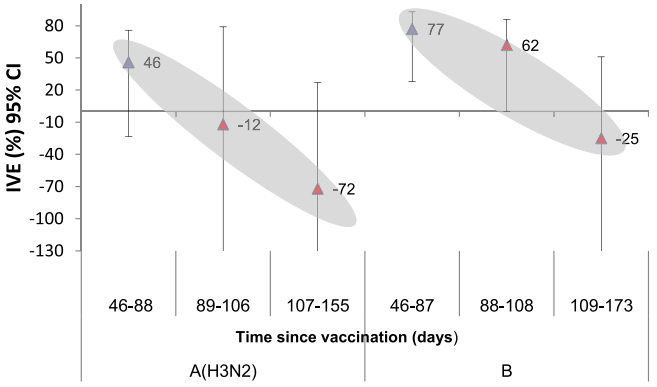


[1] IVE adjusted by sex, age (0-14; 15-64; >64 years), chronic condition, sentinel network, period of swabbing (pre-epidemic -weeks 50/2014-01/2015; epidemic - weeks 2-11/2015, post-epidemic- weeks 12-16/2015), obesity, severity, sentinel physicians visits and smoking (pregnancy not included due to low sample size: one and two pregnancies in the A(H3N2) and B analysis respectively)
[2] IVE modeled with the time since vaccination divided by tertiles and using non-vaccinated as the reference group.

**Fig. 2.** Influenza vaccine effectiveness[1] (IVE) with time since vaccination[2] (days) against A(H3N2) ($N = 440$) and B viruses ($N = 360$), cycEVA study, Spain, weeks 50/2014–16/2015.

6/2015–16/2015), however this increase was not statistically significant ($p > 0.05$).

## 4. Discussion

The IVE estimates at the end of the 2014–15 season in Spain in general population suggested a sub-optimal protective effect against influenza A(H3N2) infection, ranging between 1% in elderly to 22% in young adults. Our estimates indicated a slightly higher, but still low protective effect against A(H3N2) in target groups for vaccination, in a season dominated by a mismatched circulating A(H3N2) virus.

This low IVE against A(H3N2) seems to be in line with a considerable impact of the influenza epidemic 2014–15 in Spain, characterized by high hospitalization rates, mainly in the elderly and considerable number of reported outbreaks in nursing homes due to A(H3N2) infections [11]. In addition an excess of all-cause mortality among elderly was registered during the first 10 weeks of 2015 in Spain, as well as in other countries in Europe [11,14,15].

Previously, moderate and low IVE estimates were reported worldwide in the 2011–12 season characterized by predominant mismatched A(H3N2) virus circulation and significant impact in the mortality of elderly [6,8,16–19].

Our results at the end of the 2014–15 season suggest a possible intra-seasonal loss of vaccine protection against A(H3N2) circulating virus. The IVE point estimates obtained with SISS decreased from a significant moderate protection level (40%) in the early epidemic's phase to a non-significant value in the late phase of the epidemic. This IVE decrease was confirmed by the cycEVA study results using the same analytical approach. Moreover, our results also suggest a statistically significant decrease of the protective effect against A(H3N2) almost three months since the vaccination, with a more precise analysis within the cycEVA study. A similar loss of IVE against A(H3N2) was also described in the 2011/12 season after three and four months since influenza vaccination in United Kingdom and Navarre region (Spain), respectively [20,21].

An increase of the percentage of mismatched circulating strains or an intra-seasonal decreasing of antibodies following vaccination were among previously described possible factors related to the decrease of IVE [22–24]. At the epidemic's peak at national level, the percentage of circulating strains genetically characterized as discordant with the vaccine strain was 64%, much lower than in other Northern hemisphere settings 91% in Canada, 80% in USA and 79% in United Kingdom. This could be related to the higher interim IVE point estimates we informed in Spain 57% (95%CI: 30; 73) [25], compared with the low IVE presented mentioned settings [26–28]. At the end of the season, although the percentage of discordant strains reached 73%, this apparent increase lacked significance. We cannot conclude that the possible decrease in the IVE estimates along the season could be related to an increase of the drifted circulating A(H3N2) virus. However, there is not enough evidence allowing us to discard this possibility. It is necessary to keep in mind that the correlation of the IVE estimates with the circulating strains pattern is difficult, due to the possible biases in the selection process of the virus to be genetically characterized. A systematic approach towards virus selection for characterization would help correlating IVE estimates with genetic virus classification, providing a deeper understanding of the relation between the IVE and the degree of matching between circulating and vaccine influenza virus.

The IVE against influenza B indicated a moderate protective effect at the end of the season in all ages: 50% with a slightly higher IVE point estimate in target groups for vaccination (61%). The lowest IVE was registered for children and elderly. Previous papers presented similar IVE estimates against B infection for the general population in seasons dominated by influenza B [18,29]. In our study, the decline of vaccine protection seems to appear later (approximately 3.5 months after vaccination) and to be less pronounced than against A(H3N2) infection.

In a sensitivity analysis using surveillance data with available influenza vaccine date, we obtained similar IVE point estimates considering the vaccination status as dichotomous and taking into account the vaccination date, both against A(H3N2) as well as against B influenza viruses. Slightly higher IVE point estimates were registered for the target groups for vaccination. These results confirm that IVE estimates using national data are not biased by the current lack of influenza vaccination date within the SISS. The availability of this information, combined with the larger sample size usually available in SISS, will allow obtaining more accurate VE estimates for the future influenza seasons in Spain. Nevertheless, taking advantage of a more controlled study setting, the cycEVA study will continue playing a crucial role in confirming IVE estimates in Spain.

Our study suggested a low VE of the 2014–15 influenza vaccine against influenza A(H3N2) and a moderate protection against influenza B. Furthermore, there were suggestions of decreasing protection of influenza vaccine along the season and with time since vaccination. Efforts should be put into developing influenza vaccines capable of maintaining their protective effects through the entire influenza season.

Despite the low IVE against A(H3N2) found especially in elderly, it is important to highlight the importance of the influenza vaccination especially in population risk groups due to several reasons. Firstly there has been a late circulation of influenza B in Spain against which the vaccine had a moderate protective effect. Secondly and more important, even in conditions of low VE, the influenza vaccination still has an important impact in preventing severe outcomes mainly in the population segment considered as target group for vaccination [30].

## Conflict of interest

None.

## Authors' contributions

Alin Manuel Gherasim and Amparo Larrauri were responsible for the study design and were involved in data interpretation and manuscript preparation. Alin Manuel Gherasim undertook the

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statistical data analysis. Salvador de Mateo contributed to data analysis and interpretation. Francisco Pozo and Inmaculada Casas undertook the genetic characterization of the influenza strains and contributed with the interpretation of the virological data. All authors participated in data collection, data interpretation, contributed to the revision of the drafts manuscript and approved the final version. Authors from cycEVA team and VEVA Working group were involved in data collection, manuscript review and approved the final version.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2016.03.035.

## References

[1] Nicoll A, Ciancio B, Tsolova S, Blank P, Yilmaz C. The scientific basis for offering seasonal influenza immunisation to risk groups in Europe. Euro Surveill 2008;13(43):1–8. Available from: http://www.eurosurveillance.org/images/dynamic/EE/V13N43/art19018.pdf.
[2] World Health Organization. Vaccines against influenza. WHO position paper – November 2012. Wkly Epidemiol Rec 2012;87(47):461–76. Available from: http://www.who.int/wer/2012/wer8747.pdf.
[3] World Health Organization. Questions and answers, WHO. Recommended composition of influenza virus vaccines for use in the northern hemisphere 2014–15 influenza season and development of candidate vaccine viruses for pandemic preparedness; 2014. Available from: http://www.who.int/influenza/vaccines/virus/recommendations/201402_qanda_recommendation.pdf.
[4] Ministry of Health and Social Policy, Spain. Recommendations for influenza vaccination, season 2014–2015; 2014 (in Spanish). Available from: http://www.msssi.gob.es/profesionales/saludPublica/prevPromocion/vacunaciones/docs/Recomendaciones_vacunacion_gripe2014_2015.pdf.
[5] Larrauri A, de Mateo S. Characterisation of swabbing for virological analysis in the Spanish Influenza Sentinel Surveillance System during four influenza seasons in the period 2002–2006. Euro Surveill 2007;12(5):E5–6. Available from: http://www.eurosurveillance.org/images/dynamic/EM/V12N05/art706.pdf.
[6] Jimenez-Jorge S, de Mateo S, Delgado-Sanz C, Pozo F, Casas I, Garcia-Cenoz M, et al. Effectiveness of influenza vaccine against laboratory-confirmed influenza, in the late 2011–2012 season in Spain, among population targeted for vaccination. BMC Infect Dis 2013;13(1):441. Available from: http://www.biomedcentral.com/content/pdf/1471-2334-13-441.pdf.
[7] Savulescu C, Jimenez-Jorge S, De Mateo S, Pozo F, Casas I, Perez-Brena P, et al. Using surveillance data to estimate pandemic vaccine effectiveness against laboratory confirmed influenza A(H1N1)2009 infection: two case controls studies, Spain, season 2009–2010. BMC Public Health 2011;11:899–907.
[8] Jimenez-Jorge S, de Mateo S, Pozo F, Casas I, Garcia CM, Castilla J, et al. Early estimates of the effectiveness of the 2011/12 influenza vaccine in the population targeted for vaccination in Spain, 25 December 2011 to 19 February 2012. Euro Surveill 2012;17(12):1–6. Available from: http://www.eurosurveillance.org/images/dynamic/EE/V17N12/art20129.pdf.
[9] Jimenez-Jorge S, Pozo F, de Mateo S, Delgado-Sanz C, Casas I, Garcia-Cenoz M, et al. Influenza vaccine effectiveness in Spain 2013/14: subtype-specific early estimates using the cycEVA study. Euro Surveill 2014;19(9). Available from: http://www.eurosurveillance.org/images/dynamic/EE/V19N09/art20727.pdf.
[10] Jimenez-Jorge S, de Mateo S, Delgado-Sanz C, Pozo F, Casas I, Garcia Cenoz M, et al. Estimating influenza vaccine effectiveness in Spain using sentinel surveillance data. Euro Surveill 2015;20(28), http://dx.doi.org/10.2807/1560-7917.ES2015.20.28.21187, pii=21187.2015.
[11] Public Health Institute Carlos III. Influenza surveillance report for the 2014–2015 season in Spain; 2014. Available from: http://www.isciii.es/ISCIII/es/contenidos/fd-servicios-cientifico-tecnicos/fd-vigilancias-alertas/fd-enfermedades/pdf_2015/Informe_Vigilancia_GRIPE_2014-2015_vf_29092015.pdf (in Spanish).
[12] Jimenez-Jorge S, Savulescu C, Pozo F, de Mateo S, Casas I, Ledesma J, et al. Effectiveness of the 2010–11 seasonal trivalent influenza vaccine in Spain: cycEVA study. Vaccine 2012;30:3595–602.
[13] Niccolai LM, Ogden LG, Muehlenbein CE, Dziura JD, Vazquez M, Shapiro ED. Methodological issues in design and analysis of a matched case–control study of a vaccine's effectiveness. J Clin Epidemiol 2007;60(11):1127–31. Available from: PM:17938054.
[14] World Health Organization. Weekly Epidemiological Record. Review of the 2014–2015 influenza season in northern hemisphere; 2015 (No. 23, 2015, 90, 281–296).
[15] Molbak K, Espenhain L, Nielsen J, Tersago K, Bossuyt N, Denissov G, et al. Excess mortality among the elderly in European countries, December 2014 to February 2015. Euro Surveill 2015;20(11). Available from: PM:25811643.
[16] Kissling E, Valenciano M, Falcao J, Larrauri A, Widgren K, Pitigoi D, et al. "I-MOVE" towards monitoring seasonal and pandemic influenza vaccine effectiveness: lessons learnt from a pilot multi-centric case–control study in Europe, 2008–9. Euro Surveill 2009;14(44):1–8. Available from: http://www.eurosurveillance.org/images/dynamic/EE/V14N44/art19388.pdf.
[17] Kissling E, Valenciano M, Larrauri A, Oroszi B, Cohen J, Nunes B, et al. Low and decreasing vaccine effectiveness against influenza A(H3) in 2011/12 among vaccination target groups in Europe: results from the I-MOVE multicentre case–control study. Euro Surveill 2013;18(5). Available from: http://www.eurosurveillance.org/images/dynamic/EE/V18N05/art20390.pdf.
[18] Savulescu C, Jimenez-Jorge S, Delgado-Sanz C, de Mateo S, Pozo F, Casas I, et al. Higher vaccine effectiveness in seasons with predominant circulation of seasonal influenza A(H1N1) than in A(H3N2) seasons: test-negative case–control studies using surveillance data, Spain, 2003–2011. Vaccine 2014;32(35):4404–11.
[19] Mazick A, Gergonne B, Nielsen J, Wuillaume F, Virtanen MJ, Fouillet A, et al. Excess mortality among the elderly in 12 European countries, February and March 2012. Euro Surveill 2012;17(14). Available from: PM:22516003.
[20] Pebody R, Andrews N, McMenamin J, Durnall H, Ellis J, Thompson C, et al. Vaccine effectiveness of 2011/12 trivalent seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: evidence of waning intra-seasonal protection. Euro Surveill 2013;18(5). Available from: http://www.eurosurveillance.org/images/dynamic/EE/V18N05/art20389.pdf.
[21] Castilla J, Martinez-Baz I, Martinez-Artola V, Reina G, Pozo F, Garcia CM, et al. Decline in influenza vaccine effectiveness with time after vaccination, Navarre, Spain, season 2011/12. Euro Surveill 2013;18(5). Available from: http://www.eurosurveillance.org/images/dynamic/EE/V18N05/art20388.pdf.
[22] Matsushita M, Takeuchi S, Kumagai N, Uehara Y, Matsushita C, Arise K, et al. Prevaccination antibody titers can estimate the immune response to influenza vaccine in a rural community-dwelling elderly population. Vaccine 2012;30(6):1101–7. Available from: http://www.sciencedirect.com/science/article/pii/S0264410X11019475.
[23] Brydak LB, Machala M, Mysliwska J, Mysliwski A, Trzonkowski P. Immune response to influenza vaccination in an elderly population. J Clin Immunol 2003;23(3):214–22. Available from: http://www.springerlink.com/content/n1p8x7228tk1m006/fulltext.pdf.
[24] Uyeki TM, Zane SB, Bodnar UR, Fielding KL, Buxton JA, Miller JM, et al. Large summertime influenza A outbreak among tourists in Alaska and the Yukon Territory. Clin Infect Dis 2003;36(9):1095–102. Available from: PM:12715302.
[25] Public Health Institute Carlos III, National Center of Epidemiology. Interim estimates influenza of vaccine effectiveness in Spain, 2014/15 season; 2015 February (unpublished results).
[26] Pebody R, Warburton F, Ellis J, Andrews N, Thompson C, von Wissmann B, et al. Low effectiveness of seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: 2014/15 mid-season results. Euro Surveill 2015;20(5). Available from: PM:25677050.
[27] Flannery B, Clippard J, Zimmerman RK, Nowalk MP, Jackson ML, Jackson LA, et al. Early estimates of seasonal influenza vaccine effectiveness – United States, January 2015. MMWR Morb Mortal Wkly Rep 2015;64(1):10–5. Available from: PM:25590680.
[28] Skowronski D, Chambers C, Sabaiduc S, De Serres G, Dickinson J, Winter A, et al. Interim estimates of 2014/15 vaccine effectiveness against influenza A(H3N2) from Canada s Sentinel Physician Surveillance Network, January 2015. Euro Surveill 2015;20(4). Available from: PM:25655053.
[29] Kissling E, Valenciano M, Buchholz U, Larrauri A, Cohen J, Nunes B, et al. Influenza vaccine effectiveness estimates in Europe in a season with three influenza type/subtypes circulating: the I-MOVE multicentre case–control study, influenza season 2012/13. Euro Surveill 2014;19(6). Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20701.
[30] Castilla J, Guevara M, Martinez-Baz I, Ezpeleta C, Delfrade J, Irisarri F, et al. Enhanced estimates of the influenza vaccination effect in preventing mortality: a prospective cohort study. Medicine (Baltimore) 2015;94(30):e1240. Available from: PM:26222861.