Vaccine 38 (2020) 6524–6532

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Comparison of immunogenicity between candidate influenza A(H3N2) virus vaccine strains in Japan: A randomized controlled trial using a monovalent vaccine of A/Saitama/103/2014 (CEXP-002) and A/Hong Kong/4801/2014 (X-263)

Tetsuo Kase [a,b], Megumi Inoue [c], Saeko Morikawa [d], Hiroko Kumashiro [c], Satoshi Hiroi [d], Keiko Nakata [d], Kazuya Ito [a,b,f,g], Motoki Ishibashi [c], Tomomi Tsuru [c], Shin Irie [e], Akiko Maeda [a], Satoko Ohfuji [a,b], Wakaba Fukushima [a,b,*], Yoshio Hirota [g,h]

[a] Department of Public Health, Osaka City University Graduate School of Medicine, Osaka, Japan
[b] Research Center for Infectious Disease Sciences, Osaka City University Graduate School of Medicine, Osaka, Japan
[c] SOUSEIKAI PS Clinic, Fukuoka, Japan
[d] Department of Virology, Osaka Institute of Public Health, Osaka, Japan
[e] SOUSEIKAI, Fukuoka, Japan
[f] Healthcare Management, College of Healthcare Management, Miyama, Japan
[g] Clinical Epidemiology Research Center, SOUSEIKAI, Fukuoka, Japan
[h] College of Healthcare Management, Miyama, Japan

ARTICLE INFO

Article history:
Received 24 March 2020
Received in revised form 13 July 2020
Accepted 11 August 2020
Available online 29 August 2020

Keywords:
Influenza A(H3N2) vaccine strains
Immunogenicity
Antigenic matching
Randomized controlled trial

ABSTRACT

Background: For the 2017–18 influenza season, A/Saitama/103/2014 (CEXP-002) (Saitama strain) was antigenically more similar to prior circulating strains than A/Hong Kong/4801/2014 (X-263) (Hong Kong strain) in a ferret model and was selected as the A(H3N2) vaccine virus strain in Japan. However, the Saitama strain grew poorly, and the Japanese government switched to the Hong Kong strain, raising public concerns of poor effectiveness. To enhance understanding of the correlation between antigenicity in experimental models and immunogenicity, as a surrogate measure of vaccine effectiveness, in the human population, we compared the immunogenicity of specially-prepared single dose monovalent influenza A(H3N2) vaccines containing the Saitama or the Hong Kong strain.

Methods: A randomized controlled trial of 100 healthy adults aged 20–64 years (n = 50/group) was conducted. Virus neutralization assay was performed on sera from days 0 (pre-vaccination) and 21 (post-vaccination). Geometric mean titer (GMT), mean fold rise (MFR), seroconversion proportion (SCP), and seroprotection proportion (SPP) were calculated for vaccine strains and a representative circulating A (H3N2) virus strain (A/Osaka/188/2017).

Results: For the Hong Kong strain, post-vaccination GMT was significantly higher in the Hong Kong vaccine recipients (1:546 vs 1:260, $p < 0.01$), but MFR, SCP, and SPP were similar for both vaccine groups. For the Saitama strain, post-vaccination GMT (1:116 vs 1:61, $p = 0.01$) and SPP (86% vs 68%, $p = 0.03$) were significantly higher in the Hong Kong vaccine recipients, but MFR and SCP were similar for both vaccine groups. Against A/Osaka/188/2017, post-vaccination GMT and MFR were similar in both vaccine groups, but SCP (32% vs 4%, $p < 0.01$) and SPP (28% vs. 6%, $p < 0.01$) were significantly higher in the Hong Kong vaccine recipients.

Conclusion: The Hong Kong vaccine induced better or equivalent immunogenicity in comparison to the Saitama vaccine. Our trial showed that antigenic similarity in experimental models does not necessarily correlate with immunogenicity in the human population.

Clinical trial registration: UMIN000029293.

Abbreviations: WHO, World Health Organization; NIID, National Institute of Infectious Diseases; MHLW, Ministry of Health, Labour and Welfare; HA, hemagglutinin; HI, hemagglutination inhibition; $TCID_{50}$, 50% tissue culture infectious doses; CPE, cytopathic effect; GMT, geometric mean titer; MFR, mean fold rise; SCP, seroconversion proportion; SPP, seroprotection proportion; SD, standard deviation.

* Corresponding author at: Department of Public Health, Osaka City University Graduate School of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka 545-8585, Japan.
E-mail address: wakaba@med.osaka-cu.ac.jp (W. Fukushima).

https://doi.org/10.1016/j.vaccine.2020.08.025
0264-410X/

1. Introduction

Appropriate selection of influenza vaccine strains is critical for providing an optimal strategy for the prevention of influenza. Egg-adapted changes of influenza vaccine virus, particularly for the A(H3N2) strain, can affect virus receptor-binding and alter virus antigenicity [1], and therefore has attracted increasing attention in terms of vaccine effectiveness [2–4]. This was the case of the A/Hong Kong/4801/2014 (H3N2)-like virus, which was recommended by the World Health Organization (WHO) for the 2017–18 Northern Hemisphere influenza vaccine in March 2017 [5]. However, A/Saitama/103/2014 (CEXP-002), which was isolated in Japan and also designated a recommended A(H3N2) strain by the WHO for the 2017–18 Northern Hemisphere influenza vaccine [6], had fewer antigenic changes during egg-adaptation [7]. A/Saitama/103/2014 (CEXP-002) had no amino acid substitutions at the antigenic sites of its hemagglutinin (HA) protein but multiple substitutions in its neuraminidase protein. The National Institute of Infectious Diseases (NIID) in Japan reported that A/Saitama/103/2014 (CEXP-002) was antigenically matched to 60% of circulating A(H3N2) viruses in the 2016–17 season using post-infected ferret sera, while it was 3% for A/Hong Kong /4801/2014 (X-263) [7,8].

In Japan, only quadrivalent, inactivated, egg-cultured, split-virus influenza vaccines are available and these are only supplied by domestic vaccine manufacturers. The Ministry of Health, Labour and Welfare (MHLW) in Japan annually selects influenza vaccine strains according to the WHO's recommendation with additional consideration of the characteristics of circulating viruses around Japan, proliferation and yield of candidate vaccine strains, and the extent of antigenic matching between circulating viruses and candidate vaccine strains. For the A(H3N2) component of the 2017–18 influenza vaccine strain in Japan, the MHLW selected A/Saitama/103/2014 (CEXP-002) on May 2, 2017, with prioritization to antigenic matching. However, at an early stage of the manufacturing process for A/Saitama /103/2014 (CEXP-002), the protein recovery was too low to meet the vaccine demand in Japan: only 33% compared with production in the previous season. On July 12, 2017, the A(H3N2) vaccine strain was switched to A/Hong Kong/4801/2014 (X-263) [9], which was included in the previous season's influenza vaccine in Japan and was selected as one of the recommended strains for the 2017–18 season by the WHO, resulting in a vaccine shortage at the beginning of the 2017–18 influenza season. There was also a criticism that A/Saitama/103/2014 (CEXP-002) should had been used to provide "effective" vaccination of the public, together with the preventive use of antiviral medication to cope with potential vaccine shortage. These arguments raised a clinical question of whether the vaccine containing A/Saitama/103/2014 (CEXP-002) strain was "better" than that containing the A/Hong Kong/4801/2014 (X-263) strain.

Although vaccinations are expected to provide maximal protection when antigenic matching is high [10–12], antigenic similarity between a vaccine and circulating strains confirmed by post-infected ferret sera may not necessarily correlate with clinical vaccine effectiveness as indicated in some studies [12–14]. Furthermore, no reports have directly examined the vaccine effectiveness of multiple strains of the same subtype but with different antigenicity in single season. In this trial, we specially prepared A/Hong Kong/4801/2014 (X-263) and A/Saitama/103/2014 (CEXP-002) monovalent vaccines and compared their immunogenicity as a surrogate measure of vaccine effectiveness, to enhance our understanding of the correlation between antigenicity in experimental models and immunogenicity in the human population.

2. Materials and methods

2.1. Monovalent vaccines

Two types of A(H3N2) monovalent vaccine, the Hong Kong vaccine (lot number: HAR01) containing A/Hong Kong/4801/2014 (X-263) virus strain and the Saitama vaccine (lot number: HAT01) containing the A/Saitama/103/2014 (CEXP-002) virus strain, were produced and supplied by the Biken Foundation (Suita, Japan), which is licensed to manufacture commercial influenza vaccine products in Japan. They were produced using the same procedure as commercially available quadrivalent, inactivated, unadjuvanted, egg-cultured, split-virus influenza vaccines in Japan. These preparations contained the active ingredient equivalent to 30 μg of HA protein in 1 ml (i.e., 15 μg of HA per 0.5 ml).

2.2. Study design and subjects

This single-center, open-labelled, randomized controlled trial was conducted from September to November 2017. A total of 100 healthy Japanese adults were recruited between mid-September and early October through a volunteer panel for clinical trials managed by SOUSEIKAI, Fukuoka, Japan. Subjects were eligible if they met the following criteria: (1) aged 20–64 years, (2) not vaccinated for the 2017–18 seasonal influenza vaccine, and (3) not planning to receive a seasonal influenza vaccine until the final serum sample collection in this trial that was scheduled 3 weeks after vaccination. Exclusion criteria were: fever, severe acute illness, allergy to any component of the vaccines used for this trial, eggs, chicken, or any derivative of chicken. Subjects were also excluded if they had received any live vaccine within 27 days or any inactivated vaccine within 6 days. For recruitment, a total of 10 strata by sex and age were created *a priori* and a fixed number of subjects was recruited for each stratum (25 subjects for 20–29 /30–39 year groups, 20 subjects for 40–49/50–59 year groups, and 10 subjects for the 60–64 year group, in both sexes).

Subjects were randomly allocated to receive the Hong Kong vaccine (n = 50) or the Saitama vaccine (n = 50) by blocked randomization with a block size of 4. We did not employ stratified randomization for allocation. Sample size calculations were not conducted in advance, because this trial involved monovalent influenza vaccines that were specially prepared under the request of the MHLW Japan and thus financial resources for the trial were limited.

Written consent was obtained from all subjects. This study was performed in accordance with the Declaration of Helsinki, and the study protocol was approved by the ethics committee at SOUSEIKAI PS Clinic (No. K-144), Osaka City University Graduate School of Medicine (No. 3276) and Osaka Institute of Public Health (No. 1711-01).

2.3. Information collection, vaccination and serum sample collection

At recruitment, a self-administrated questionnaire was used to collect the subjects' baseline information including sex, age, height, body weight, smoking status, history of influenza vaccination and influenza diagnosis during the previous 3 seasons. Subjects received either the Hong Kong vaccine or the Saitama vaccine *via* a 0.5 ml subcutaneous injection into the extensor side of the upper arm opposite the dominant arm using a 27G needle. The subjects were observed for 30 min following vaccination to monitor allergic reactions and to respond and treat them quickly if they occurred.

To assess the safety of each vaccine, information on adverse reactions (local and systemic) occurring within one week following vaccination was collected using a self-administered questionnaire.

Solicited adverse reactions included 6 localized reactions (redness, swelling, induration, pain, itching, and heat sensation) and 7 systemic reactions (fever, fatigue, myalgia/arthralgia, headache, nausea, diarrhea, and rash). Information on fever was collected using 8 categories (no fever, 37.0–39.9 °C by 0.5 °C, and ≥40.0 °C). Other local/systemic reactions were reported with 3 categories of severity (mild, moderate and severe). Definitions of "mild/moderate/severe" reactions were as follows: "within a few centimeters/within the elbow or shoulder/spreading over the elbow or shoulder" for redness, swelling, and induration; "no requirement for medication/requirement for medication/requirement for medication with other symptoms" for rash; and "no influence on daily life/influence on daily life/substantial influence on daily life" for other reactions. Subjects were also asked to provide any unsolicited adverse reactions or severe adverse events following immunization in an open-ended form.

On day 0 (i.e., pre-vaccination) and day 21 after vaccination, blood samples were collected and centrifuged to separate supernatant and precipitation. Sample collection was completed in early November, before the start of the 2017–18 influenza season in Japan (20 November 2017), as declared by the NIID Japan. The serum samples were then stored at −20 °C until the neutralization test was performed.

2.4. Measurement of antibody titers

Antigenic characterization of recent circulating A(H3N2) viruses using an hemagglutination inhibition (HI) assay is technically difficult because many viruses do not agglutinate red blood cells. Indeed, virus neutralization assays have supplemented HI assays for the antigenic characterization of viruses [5]. In this trial, neutralization antibody titers were measured for each of the virus strains contained in the vaccines and for a currently circulating strain [15–17]. In brief, serum samples were treated with a receptor destroying enzyme (Denka Seiken, Tokyo, Japan) and inactivated for 60 min at 56 °C to remove non-specific inhibitors. A 1:10 dilution of treated sera was prepared with phosphate-buffered saline. Two-fold serial dilutions of sera were mixed with one hundred 50% tissue culture infectious doses ($TCID_{50}$) of the influenza viruses and were preincubated for 60 min at 37 °C. The mixtures were inoculated onto MDCK-SIAT1 cells (KAC, Kyoto, Japan) in duplicate in 96-well plates and then incubated for 60 min at 37 °C. The supernatant was removed and Dulbecco's modified Eagle's medium–high glucose (Sigma-Aldrich D6429) with 3 μg/ml of acetylated trypsin (Sigma-Aldrich T6763) was added into the wells for influenza viral growth. Then, the plates were incubated for 4 days at 35 °C in a CO_2 incubator. To determine the neutralizing activity of the test sera, the cytopathic effect (CPE) was observed using an inverted microscope after the cells had been stained with 1% amid black solution to obtain the best image. The neutralizing titer was defined as the reciprocal of the highest serum dilution at which the infectivity of 100 $TCID_{50}$ of the challenged virus was neutralized in 100% of the wells. The neutralizing titers were calculated as the average of duplicates. Negative samples were assigned a titer of 1:5.

A/Hong Kong/4801/2014 and A/Saitama /103/1014 virus strains were provided by the Influenza Virus Research Center, NIID, Tokyo, Japan. A/Osaka/188/2017, belonging to the 2C1a sub-clade of A(H3N2) strains, was isolated with MDCK-SIAT1 cells in Osaka prefecture Japan in December 2017.

2.5. Statistical analyses

Four types of measurements were used to compare the immunogenicity between the groups: geometric mean titer (GMT), mean fold rise (MFR), seroconversion proportion (SCP, ≥4-fold rise), and seroprotection proportion (SPP, post-vaccination titer ≥1:40). For data processing, titers <1:10 and titers ≥1:1280 were regarded as 1:5 and 1:1280, respectively. Reciprocal antibody titers were analyzed after logarithmic transformation, followed by antilogarithm to present the values in the original scale. We also compared the occurrence of adverse reactions. Differences between the groups were assessed using the Wilcoxon rank sum test, chi-squared test, or Fisher's exact test as appropriate.

Immunogenicity was also evaluated using stratification by age. Considering the potential effect of HA imprinting, we categorized the subjects according to the following three age groups: 20–39 years (born after 1977, with first exposure to A[H1N1] or A[H3N2]), 40–48 years (born between 1968 and 1977, with first exposure to A[H3N2]), and 49–64 years (born before 1968, with first exposure to A[H2N2] or A[H1N1]) [18].

All hypothesis testing was conducted assuming a 0.05 significance level and a two-sided alternative hypothesis. SAS software version 9.3 (SAS Institute, Inc., Cary, NC) was used for all analyses.

3. Results

The distributions of subject background characteristics including sex, age, body weight, body mass index, smoking status, antibody titers prior to vaccination, history of influenza vaccination, and history of influenza diagnosis were similar between the Hong Kong vaccine group and the Saitama vaccine group (Table 1). No significant differences were observed.

Within 48 h after vaccination, many mild local adverse reactions were observed in both groups, while redness, pain or itching were observed only in the Saitama vaccine group from 48 h to 1 week (Fig. 1). No subjects reported moderate or severe local reactions. Three subjects (6%) in the Saitama vaccine group reported a fever of 37.0–37.4 °C within 48 h after vaccination. With regard to other systemic reactions, a very small number of subjects experienced mild reactions including fatigue, headache or diarrhea (Fig. 2). No subjects reported moderate or severe systemic reactions, except for one subject (2%) in the Saitama vaccine group who reported moderate fatigue from 48 h to 1 week following vaccination. There were no significant differences between the groups.

Neutralization antibody titers in pre- and post-vaccination groups are shown in Fig. 3, where all data points are plotted. Table 2 shows four types of measurements of immunogenicity against three kinds of strains in the two vaccine groups. For A/Hong Kong/4801/2014, the post-vaccination GMT was significantly higher for the Hong Kong vaccine recipients (1:546 vs 1:260, $p < 0.01$), but the MFR (5.5 vs 4.5, $p = 0.34$), SCP (50% vs 40%, $p = 0.31$), and SPP (96% vs 92%, $P = 0.68$) were similar for both vaccine groups. For A/Saitama/103/2014, the post-vaccination GMT (1:116 vs 1:61, $p = 0.01$) and SPP (86% vs 68%, $p = 0.03$) were significantly higher in the Hong Kong vaccine recipients, but the MFR (5.3 vs 4.2, $p = 0.60$) and SCP (50% vs 46%, $p = 0.69$) were similar for both vaccine groups. Against the A/Osaka/188/ 2017 virus, the post-vaccination GMT (1:17 vs 1:9, $p = 0.20$) and MFR (2.7 vs 1.3, $p = 0.16$) were similar in both vaccine groups, but the SCP (32% vs 4%, $p < 0.01$) and SPP (28% vs 6%, $p < 0.01$) were significantly higher for the Hong Kong vaccine recipients. The Hong Kong vaccine recipients also showed a higher SPP with a cut-off value of 1:20 (36% vs 10%, $p < 0.01$).

Stratified analyses by age groups are shown in Table 3. The overall findings were similar to those in all subjects, although the statistical significance was lost because of the small number of subjects in each group. Post-vaccination GMT against A/Hong Kong/4801/2014 or A/Saitama/103/2014 and SPP against A/Saitama/103/2014 was higher in the Hong Kong vaccine recipients

Table 1
Characteristics of the subjects.

	Hong Kong vaccine group n (%) or mean [SD]		Saitama vaccine group n (%) or mean [SD]	
Male sex	25	(50)	25	(50)
Age (years)				
20–29	13	(26)	12	(24)
30–39	14	(28)	11	(22)
40–49	9	(18)	11	(22)
50–59	8	(16)	12	(24)
60–64	6	(12)	4	(8)
Age (years)				
20–39 [born after 1977]	27	(54)	23	(46)
40–48 [born between 1968 and 1977]	8	(16)	9	(18)
49–64 [born before 1968]	15	(30)	18	(36)
Body weight (kg)	61.9	[11.7]	61.9	[13.8]
Body mass index (kg/m^2)	22.5	[3.4]	22.7	[3.4]
Smoking status				
Never	35	(70)	37	(74)
Former	4	(8)	5	(10)
Current	11	(22)	8	(16)
Pre-titer				
A/Hong Kong/4801/2014				
<10	5	(10)	8	(16)
10	3	(6)	5	(10)
20	2	(4)	7	(14)
≥40	40	(80)	30	(60)
A/Saitama/103/2014				
<10	15	(30)	22	(44)
10	5	(10)	4	(8)
20	11	(22)	11	(22)
≥40	19	(38)	13	(26)
A/Osaka/188/2017				
<10	40	(80)	41	(82)
10	7	(14)	5	(10)
20	2	(4)	2	(4)
≥40	1	(2)	2	(4)
Previous influenza vaccination				
2014–15 season				
No	37	(74)	33	(66)
Yes	9	(18)	9	(18)
Unknown	4	(8)	8	(16)
2015–16 season				
No	40	(80)	35	(70)
Yes	8	(16)	10	(20)
Unknown	2	(4)	5	(10)
2016–17 season				
No	41	(82)	40	(80)
Yes	7	(14)	8	(16)
Unknown	2	(4)	2	(4)
Previous influenza diagnosis				
2014–15 season				
No	38	(76)	44	(88)
Type A	1	(2)	0	(0)
Type B	0	(0)	0	(0)
Types unknown	7	(14)	5	(10)
Unknown	4	(8)	1	(2)
2015–16 season				
No	44	(88)	46	(92)
Type A	2	(4)	2	(4)
Type B	0	(0)	0	(0)
Types unknown	2	(4)	1	(2)
Unknown	2	(4)	1	(2)
2016–17 season				
No	45	(90)	44	(48)
Type A	2	(4)	2	(4)
Type B	0	(0)	1	(2)
Types unknown	2	(4)	3	(6)
Unknown	1	(2)	0	(0)

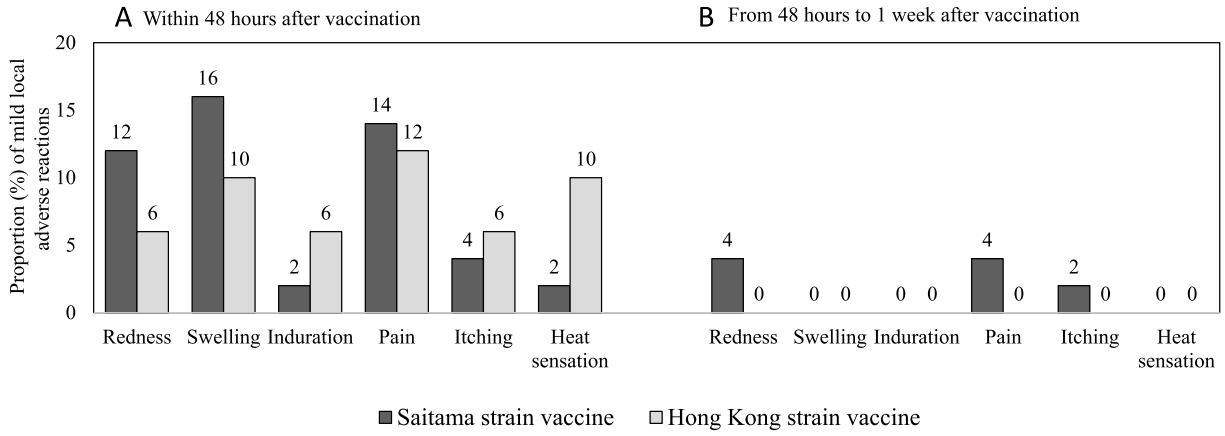

Fig. 1. Proportion (%) of mild local adverse reactions following vaccination within 48 h (A) and from 48 h to 1 week (B) after vaccination. Mild local adverse reactions were defined as "within a few centimeters" for redness, swelling and induration and "no influence on daily life" for pain, itching and heat sensation.

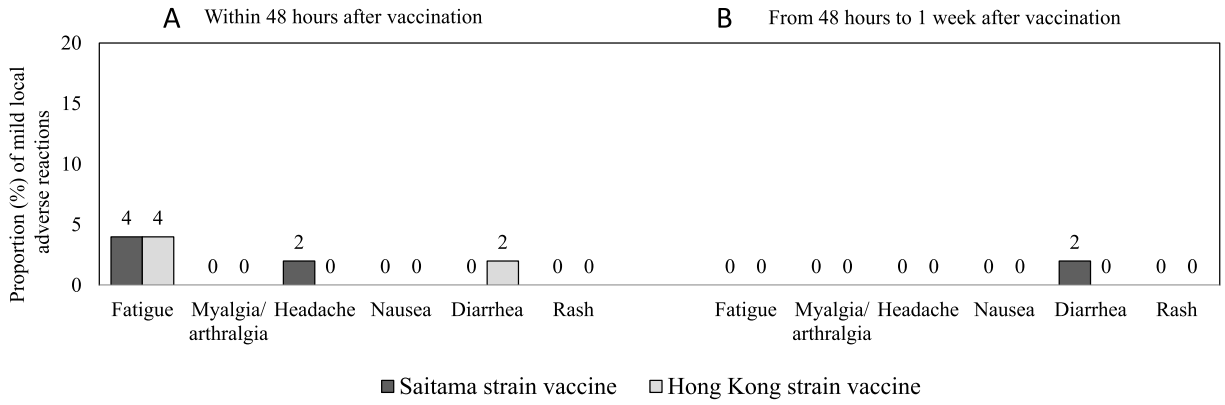

Fig. 2. Proportion (%) of mild systemic adverse reactions following vaccination within 48 h (A) and from 48 h to 1 week (B) after vaccination. Mild systemic adverse reactions were defined as "no influence on daily life" for fatigue, myalgia/arthralgia, headache, nausea, and diarrhea, and "no requirement for medication" for rash.

regardless of age. Against the A/Osaka/188/2017 virus, the Hong Kong vaccine recipients showed a higher SCP and SPP in each age group. Focusing on immunogenicity in the Hong Kong vaccine recipients, higher responses were observed in subjects aged 40–48 years (born between 1968 and 1977, with first exposure to A [H3N2]) and 49–64 years (born before 1968, with first exposure to A[H2N2] or A[H1N1]) than those aged 20–39 years (born after 1977, with first exposure to A[H1N1] or A[H3N2]).

4. Discussion

Our trial showed that the Hong Kong vaccine induced good immune responses not only against its homosubtypic, A/Hong Kong/4801/2014, but also against the heterosubtypic, A/Saitama/103/2014. Antibody responses against A/Saitama/103/2014 induced by the Hong Kong vaccine were similar to or better than those induced by the Saitama vaccine. Furthermore, the Hong Kong vaccine exhibited a superior ability to induce antibody against a seasonal wild-type strain (i.e., A/Osaka/188/2017) compared with the Saitama vaccine. Overall, the Hong Kong vaccine showed better immunogenicity than the Saitama vaccine against a broader range of influenza virus strains. Similar findings were also observed after stratification by age. Our trial clearly showed that antigenic similarity between vaccine strains and circulating strains in the previous season (i.e., predicted epidemic strains in the upcoming season) in experimental models does not necessarily correlate with immunogenicity in the human population, probably because humans have previous experience of virus exposure or vaccination [19–22].

Although comparing the clinical effectiveness, not immunogenicity, of the Saitama vaccine and the Hong Kong vaccine in a human population would be desirable to obtain direct evidence, this is impossible under real-world settings because A/Saitama/103/2014 (CEXP-002) was not included in the commercially distributed influenza vaccine during the 2017–18 season in Japan. The serum antibody titer is not a direct measurement of vaccine effectiveness, rather it is a surrogate variable. Nevertheless, because it is expected that strong immunogenicity induced by a vaccine strain induces a moderate level of antibodies against circulating strains in the influenza season [23], excellent immunogenicity may predict high influenza vaccine effectiveness [24].

Significantly higher GMT or SPP were observed in Hong Kong vaccine recipients compared with Saitama vaccine recipients against their homosubtypic or heterosubtypic vaccine strain. However, both vaccines induced a sufficient antibody response in accord with the international licensing criteria. The European Medicines Agency, Committee for Medicinal Products for Human Use (CHMP) has established criteria as follows: MFR > 2.5, SCP > 40%, and SPP > 70% for adults aged 18–60 years when HI titers are assayed against the prototype strain of the vaccine [25]. Our study vaccines almost met these three criteria, although neutralization antibody titers, not HI titers, were measured. To explore the possible reason for the different immunogenicity between the

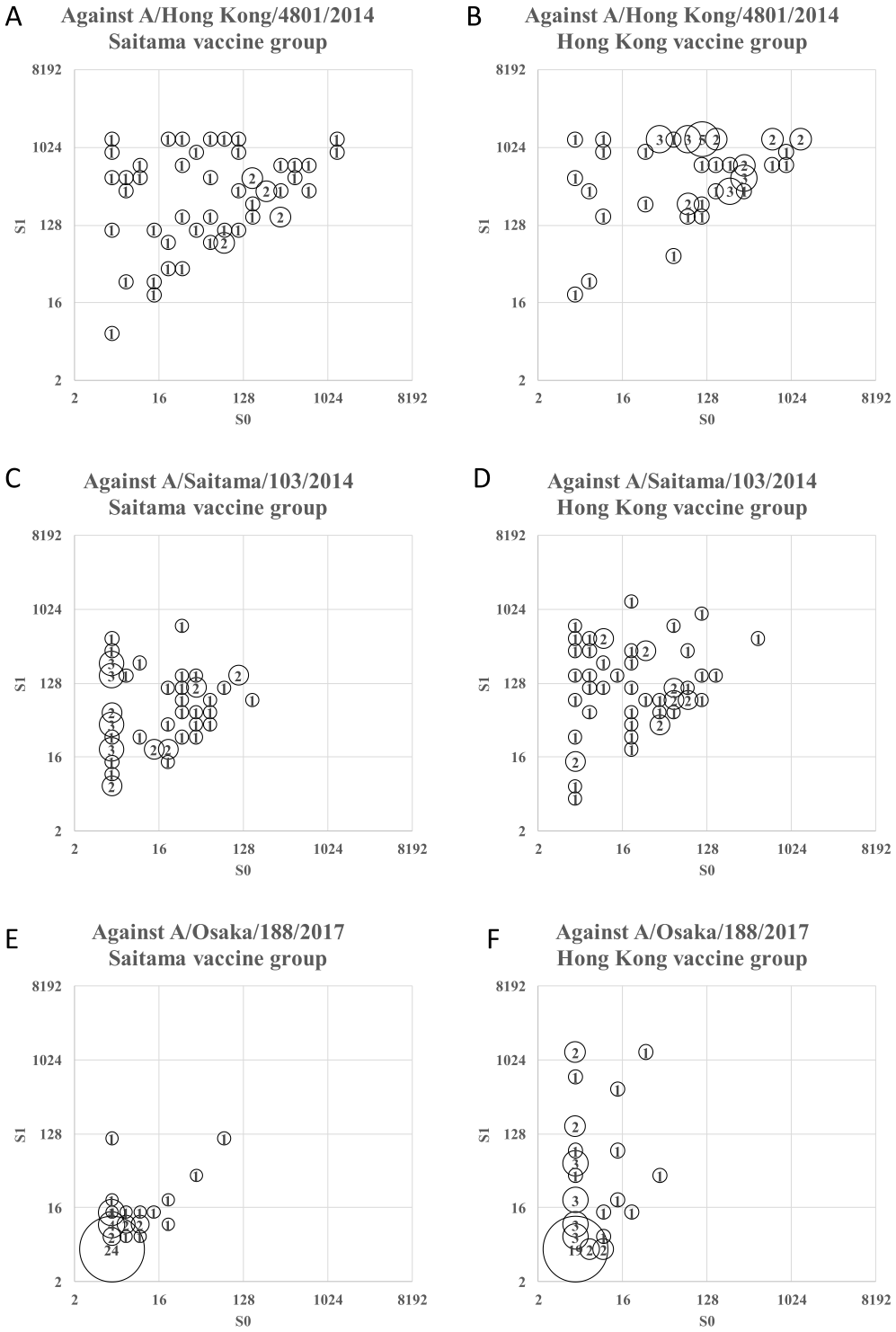

Fig. 3. Antibody titers in two vaccine groups. All data points for neutralization antibody titers in pre- and post-vaccination groups are shown as scatter dot plots. Against A/Hong Kong/4801/2014 in the Saitama vaccine group (A) and Hong Kong vaccine group (B), against A/Saitama/103/2014 in the Saitama vaccine group (C) and the Hong Kong vaccine group (D), and against A/Osaka/188/2017 in the Saitama vaccine group (E) and the Hong Kong vaccine group (F) are shown. S0 on X axes and S1 on Y axes indicate day 0 (pre) and day 21 (post) vaccination, respectively.

vaccines, HA sequences of the two vaccine strains and the circulating strain were evaluated (Supplementary Fig. 1). Amino acid changes (T160K or L194P substitution at an antigenic site in the HA of recent H3N2 viruses) introduced through egg passaging affected antigenicity. A/Hong Kong/4801/2014 (X-263) has both T160K and L194P substitutions, while A/Saitama/103/2014 (CEXP-002) only has the T160K substitution. No changes were found in the circulating strain of A/Osaka/188/2017 at both posi-

Table 2
Neutralizing antibody titers against three influenza virus strains.

	GMT			SCP (%)	SPP (%)	
	S0	S1	MFR (S1/S0)	(S1/S0 ≥ 4)	(S1 ≥ 1:40)	(S1 ≥ 1:20)
Against A/Hong Kong/4801/2014						
Hong Kong vaccine group	99	546	5.5	50	96	–
Saitama vaccine group	57	260	4.5	40	92	–
P value*	–	<0.01	0.34	0.31	0.68	–
Against A/Saitama/103/2014						
Hong Kong vaccine group	22	116	5.3	50	86	–
Saitama vaccine group	15	61	4.2	46	68	–
P value*	–	0.01	0.60	0.69	0.03	–
Against A/Osaka/188/2017						
Hong Kong vaccine group	6	17	2.7	32	28	36
Saitama vaccine group	6	9	1.3	4	6	10
P value*	–	0.20	0.16	<0.01	<0.01	<0.01

GMT: geometric mean titer; MFR: mean fold rise; SCP: seroconversion proportion; SPP: seroprotection proportion; S0: sera at pre-vaccination; S1: sera at post-vaccination.
* Wilcoxon rank sum test, chi-squared test or Fisher's exact test, as appropriate.

Table 3
Age-stratified neutralizing antibody titers against three influenza virus strains.

	GMT			SCP (%)	SPP (%)	
	S0	S1	MFR (S1/S0)	(S1/S0 ≥ 4)	(S1 ≥ 1:40)	(S1 ≥ 1:20)
Against A/Hong Kong/4801/2014						
20–39 years old [born after 1977]						
Hong Kong vaccine group (n = 27)	146	435	3.0	33	96	–
Saitama vaccine group (n = 23)	113	315	2.8	30	100	–
P value*	–	0.21	0.43	>0.99	>0.99	–
40–48 years old [born between 1968 and 1977]						
Hong Kong vaccine group (n = 8)	77	905	11.8	75	100	–
Saitama vaccine group (n = 9)	34	132	3.8	33	78	–
P value*	–	0.02	0.33	0.15	0.47	–
49–64 years old [born before 1968]						
Hong Kong vaccine group (n = 15)	55	625	11.3	67	93	–
Saitama vaccine group (n = 18)	31	285	9.2	56	89	–
P value*	–	0.07	0.77	0.72	>0.99	–
Against A/Saitama/103/2014						
20–39 years old [born after 1977]						
Hong Kong vaccine group (n = 27)	29	81	2.8	33	85	–
Saitama vaccine group (n = 23)	22	60	2.7	35	70	–
P value*	–	0.23	0.70	>0.99	0.30	–
40–48 years old [born between 1968 and 1977]						
Hong Kong vaccine group (n = 8)	15	190	12.3	75	88	–
Saitama vaccine group (n = 9)	11	38	3.6	33	44	–
P value*	–	0.04	0.18	0.15	0.13	–
49–64 years old [born before 1968]						
Hong Kong vaccine group (n = 15)	16	168	10.8	67	87	–
Saitama vaccine group (n = 18)	10	78	8.0	67	78	–
P value*	–	0.08	0.58	>0.99	0.66	–
Against A/Osaka/188/2017						
20–39 years old [born after 1977]						
Hong Kong vaccine group (n = 27)	7	11	1.7	22	15	30
Saitama vaccine group (n = 23)	8	10	1.3	4	13	13
P value*	–	0.96	0.76	0.11	>0.99	0.19
40–48 years old [born between 1968 and 1977]						
Hong Kong vaccine group (n = 8)	6	38	5.9	50	50	50
Saitama vaccine group (n = 9)	7	7	1.1	0	0	11
P value*	–	0.15	0.25	0.03	0.03	0.13
49–64 years old [born before 1968]						
Hong Kong vaccine group (n = 15)	6	22	4.0	40	40	40
Saitama vaccine group (n = 18)	5	8	1.5	6	0	6
P value*	–	0.28	0.24	0.03	<0.01	0.03

GMT: geometric mean titer; MFR: mean fold rise; SCP: seroconversion proportion; SPP: seroprotection proportion; S0: sera at pre-vaccination; S1: sera at post-vaccination.
* Wilcoxon rank sum test for GMT and MFR, Fisher's exact test for SCP and SPP.

tions because it was isolated by cell-culture. Because a T160K substitution in the glycosylation site was more important in terms of antigenicity [4], it is reasonable to suggest that the two vaccine strains in this trial were genetically similar. Our findings indicate that some influenza vaccine strains can maintain antigenicity even though substantial egg-adapted changes occur. This interpretation might be in line with a report where the WHO recommended A/Hong Kong/4801/2014-like viruses for the A(H3N2) vaccine strain in the 2017–18 season because most recent isolated A(H3N2) viruses were inhibited by ferret antisera raised against cell culture-propagated reference viruses [5].

Our trial with unique monovalent vaccines provided a supplemental opportunity to evaluate the phenomenon of imprinting, or "original antigenic sin", indicating that the development of immunity against pathogens is shaped by the first exposure to a related pathogen [26]. Regarding the possible imprinting of influenza virus using human sera, measurable differences in antibody response according to the potential first exposure to influenza A virus subtypes (A[H1N1], A[H2N2] and A[H3N2]) was reported [27], whereas another study reported a greater response by monovalent A(H1N1)pdm09 vaccine against the A(H1N1) pdm09 virus rather than against the A(H1N1) virus that circulated during the childhood of each subject [28]. Similar to the latter finding, our subgroup analysis did not show a clear effect of imprinting. The monovalent Hong Kong vaccine-induced antibody response against three A(H3N2) viruses was generally high in subjects aged 40–48 years (first exposure: A[H3N2]) and aged 49–64 years (first exposure: A[H1N1] or A[H2N2]). Overall, the lowest immunogenicity was in the youngest age group (i.e., 20–39 years, with first exposure to A[H1N1] or A[H3N2]), although they almost met the CHMP criteria. This indicates the importance of factors other than the first viral exposure.

Almost all adverse reactions were mild and their frequency was similar between the groups. Our safety profiles cannot be directly compared with that of conventional seasonal inactivated vaccines because the vaccines in this trial were monovalent. However, our frequency was similar to or lower than those in previous clinical trials in which a monovalent, inactivated, unadjuvanted, split-virus A(H1N1) pdm09 vaccine with 15 μg of HA per 0.5 ml was administered intramuscularly [29–32], except for the frequency of redness or swelling ($\leq$16%) which may attribute to the subcutaneous injection route of administration in our trial. Overall, our study vaccines were well tolerated.

Strengths of our study included the unique use of specially prepared monovalent influenza vaccines, which enabled us to evaluate a real-world-like reaction of human antibody responses by a single vaccine strain. Despite the constraints of time and manufacturing processes, the trial was completed before the influenza epidemic, leading to a straightforward interpretation of the results without the effect of the natural infection of influenza.

Several limitations should be considered. Because study participants were only healthy adults aged 20–64 years, the representativeness of the findings is limited. Children, adolescents aged 19 years or younger, and the elderly aged 65 or more, who may have different immunological status from the study population, were not evaluated in this trial. The size of the clinical trial was small because of the feasibility of the study and budget issues. Antibody responses against circulating strains did not meet the CHMP criteria. Because the international licensing criteria does not require evaluation against circulating strains and few studies have examined such immunogenicity, it cannot be determined whether the induced antibodies against circulating strains in this trial were sufficient. We used only one isolated strain belonging to the 2C1a sub-clade to determine immunogenicity against circulating viruses consisting of many sub-clades. Of note, vaccinated human sera may react differently to viruses belonging to other sub-clades. Finally, information on the total protein or neuraminidase content of the study vaccine was not available. This may affect the immunogenicity of the vaccines despite standardization by HA content.

5. Conclusions

The Hong Kong vaccine showed good immunogenicity, not only against its homosubtypic, A/Hong Kong/4801/2014, but also against the heterosubtypic, A/Saitama/103/2014. Moreover, the Hong Kong vaccine showed better immunogenicity against a seasonal wild-type strain compared with the Saitama vaccine. Our findings demonstrate that antigenic similarity between a predicted influenza epidemic strain and the influenza vaccine strain in experimental animal models does not necessarily correlate with immunogenicity in a human population. The same explanation might be used for vaccine effectiveness, which should be investigated in further studies.

CRediT authorship contribution statement

Tetsuo Kase: Conceptualization, Methodology, Investigation, Data curation, Validation, Writing - original draft, Writing - review & editing. **Megumi Inoue:** Project administration, Methodology, Investigation. **Saeko Morikawa:** Methodology, Investigation. **Hiroko Kumashiro:** Project administration, Resources. **Satoshi Hiroi:** Methodology, Investigation. **Keiko Nakata:** Methodology, Investigation. **Kazuya Ito:** Formal analysis. **Motoki Ishibashi:** Resources. **Tomomi Tsuru:** Resources. **Shin Irie:** Resources, Supervision. **Akiko Maeda:** Supervision. **Satoko Ohfuji:** Formal analysis, Supervision. **Wakaba Fukushima:** Conceptualization, Project administration, Formal analysis, Data curation, Validation, Writing - original draft, Writing - review & editing. **Yoshio Hirota:** Conceptualization, Funding acquisition, Data curation, Validation, Supervision.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: 'TK has received a reward for advisor tasks from The Biken Foundation from April 2016 to March 2018. The other authors have nothing to declare'.

Acknowledgements

We thank Dr. Shinji Watanabe and Dr. Takato Odagiri in the Influenza Virus Research Center, NIID, Tokyo, Japan, for providing vaccine strains. We also thank Dr. Kazushi Motomura and Dr. Yoshinobu Okuno in the Osaka Institute of Public Health, Osaka, Japan, for allowing us to use laboratory equipment for the neutralization assays. We thank all the staff at the SOUSEIKAI PS Clinic, Fukuoka, Japan, for helping with information collection, vaccination and serum sample collection.

Funding

This study was funded by a research grant for Research on Emerging/Re-emerging Infectious Diseases and Promoting Vaccination Policy from the Ministry of Health, Labour and Welfare, Japan (H29-SHINKOGYOSEI-SHITEI-003).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2020.08.025.

References

[1] Parker L, Wharton SA, Martin SR, Cross K, Lin Y, Liu Y, et al. Effects of egg-adaptation on receptor-binding and antigenic properties of recent influenza A (H3N2) vaccine viruses. J Gen Virol 2016;97:1333–44.

[2] Skowronski DM, Janjua NZ, De Serres G, Sabaiduc S, Eshaghi A, Dickinson JA, et al. Low 2012–13 influenza vaccine effectiveness associated with mutation in the egg-adapted H3N2 vaccine strain not antigenic drift in circulating viruses. PLoS ONE 2014;9:e92153.

[3] Wu NC, Zost SJ, Thompson AJ, Oyen D, Nycholat CM, McBride R, et al. A structural explanation for the low effectiveness of the seasonal influenza H3N2 vaccine. PLoS Pathog 2017;13:e1006682.

[4] Zost SJ, Parkhouse K, Gumina ME, Kim K, Diaz Perez S, Wilson PC, et al. Contemporary H3N2 influenza viruses have a glycosylation site that alters binding of antibodies elicited by egg-adapted vaccine strains. Proc Natl Acad Sci U S A 2017;114:12578–83.

[5] World Health Organization. Recommended composition of influenza virus vaccines for use in the 2017–2018 northern hemisphere influenza season. Available at: https://www.who.int/influenza/vaccines/virus/recommendations/201703_recommendation.pdf?ua=1 [accessed March 16, 2020].

[6] World Health Organization. Influenza A(H3N2) egg-derived candidate vaccine viruses for development and production of vaccines for use in the 2017–2018 northern hemisphere influenza season. Available at: https://www.who.int/influenza/vaccines/virus/candidates_reagents/summary_a_h3n2_cvv-egg_nh1718.pdf?ua=1 [accessed March 16, 2020].

[7] Kuwahara T, Takashita E, Fujisaki S, Shirakura M, Nakamura K, Kishida N, et al. Isolation of an egg-adapted influenza A(H3N2) virus without amino acid substitutions at the antigenic sites of its hemagglutinin. Jpn J Infect Dis 2018;71:234–8.

[8] National Expert Committee for Research Development and Supply for Vaccines, Ministry of Health, Labour and Welfare, Japan. Influenza vaccines for 2017–18 season (Material 1 at the meeting held in August 25, 2017) [in Japanese]. Available at: https://www.mhlw.go.jp/file/05-Shingikai-10601000-Daijinkanboukouseikagakuka-Kouseikagakuka/0000175511.pdf [accessed March 16, 2020].

[9] Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare, Japan. Candidate vaccine viruses for the influenza vaccines to be used in the 2017/18 season, Japan—description of the selection process. Infect Agent Surveill Rep 2017;38(11):225 [in Japanese]. Available at: https://www.niid.go.jp/niid/images/idsc/iasr/38/453.pdf [accessed March 16, 2020].

[10] Belongia EA, Kieke BA, Donahue JG, Greenlee RT, Balish A, Foust A, et al. Effectiveness of inactivated influenza vaccines varied substantially with antigenic match from the 2004–2005 season to the 2006–2007 season. J Infect Dis 2009;199:159–67.

[11] Darvishian M, Dijkstra F, van Doorn E, Bijlsma MJ, Donker GA, de Lange MM, et al. Influenza vaccine effectiveness in the Netherlands from 2003/2004 through 2013/2014: the importance of circulating influenza virus types and subtypes. PLoS ONE 2017;12:e0169528.

[12] Tricco AC, Chit A, Soobiah C, Hallett D, Meier G, Chen MH, et al. Comparing influenza vaccine efficacy against mismatched and matched strains: a systematic review and meta-analysis. BMC Med 2013;11:153.

[13] Dean AS, Moffatt CR, Rosewell A, Dwyer DE, Lindley RI, Booy R, et al. Incompletely matched influenza vaccine still provides protection in frail elderly. Vaccine 2010;28:864–7.

[14] Kelly HA, Sullivan SG, Grant KA, Fielding JE. Moderate influenza vaccine effectiveness with variable effectiveness by match between circulating and vaccine strains in Australian adults aged 20–64 years, 2007–2011. Influenza Other Respir Viruses 2013;7:729–37.

[15] Allwinn R, Geiler J, Berger A, Cinatl J, Doerr HW. Determination of serum antibodies against swine-origin influenza A virus H1N1/09 by immunofluorescence, haemagglutination inhibition, and by neutralization tests: how is the prevalence rate of protecting antibodies in humans?. Med Microbiol Immunol 2010;199:117–21.

[16] Frank AL, Puck J, Hughes BJ, Cate TR. Microneutralization test for influenza A and B and parainfluenza 1 and 2 viruses that uses continuous cell lines and fresh serum enhancement. J Clin Microbiol 1980;12:426–32.

[17] Kishida N, Fujisaki S, Yokoyama M, Sato H, Saito R, Ikematsu H, et al. Evaluation of influenza virus A/H3N2 and B vaccines on the basis of cross-reactivity of postvaccination human serum antibodies against influenza viruses A/H3N2 and B isolated in MDCK cells and embryonated hen eggs. Clin Vaccine Immunol 2012;19:897–908.

[18] Gostic KM, Ambrose M, Worobey M, Lloyd-Smith JO. Potent protection against H5N1 and H7N9 influenza via childhood hemagglutinin imprinting. Science 2016;354(6313):722–6.

[19] Skowronski DM, Chambers C, Sabaiduc S, De Serres G, Winter AL, Dickinson JA, et al. Beyond antigenic match: possible agent-host and immuno-epidemiological influences on influenza vaccine effectiveness during the 2015–2016 season in Canada. J Infect Dis 2017;216:1487–500.

[20] Darvishian M, Bijlsma MJ, Hak E, van den Heuvel ER. Effectiveness of seasonal influenza vaccine in community-dwelling elderly people: a meta-analysis of test-negative design case-control studies. Lancet Infect Dis 2014;14:1228–39.

[21] Lewnard JA, Cobey S. Immune history and influenza vaccine effectiveness. Vaccines (Basel) 2018(6). pii: E28.

[22] Van Epps P, Tumpey T, Pearce MB, Golding H, Higgins P, Hornick T, et al. Preexisting immunity, not frailty phenotype, predicts influenza postvaccination titers among older veterans. Clin Vaccine Immunol 2017;24. pii: e00498-16.

[23] Hiroi S, Morikawa S, Nakata K, Maeda A, Kanno T, Irie S, et al. Trivalent influenza vaccine-induced antibody response to circulating influenza A(H3N2) viruses in 2010/11 and 2011/12 seasons. Hum Vaccin Immunother 2015;11:386–90.

[24] Cobey S, Gouma S, Parkhouse K, Chambers BS, Ertl HC, Schmader KE, et al. Poor immunogenicity, not vaccine strain egg adaptation, may explain the low H3N2 influenza vaccine effectiveness in 2012–2013. Clin Infect Dis 2018;67:327–33.

[25] Committee for Proprietary Medicinal Products (CPMP): Note for guidance on harmonisation of requirements for influenza vaccines. (CPMP/BWP/214/96). 1997. Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/note-guidance-harmonisation-requirements-influenza-vaccines_en.pdf [accessed July 2, 2020].

[26] Zhang A, Stacey HD, Mullarkey CE, Miller MS. Original antigenic sin: how first exposure shapes lifelong anti-influenza virus immune responses. J Immunol 2019;202(2):335–40.

[27] Nachbagauer R, Choi A, Hirsh A, Margine I, Iida S, Barrera A, et al. Defining the antibody cross-reactome directed against the influenza virus surface glycoproteins. Nat Immunol 2017;18(4):464–73.

[28] O'Donnell CD, Wright A, Vogel L, Boonnak K, Treanor JJ, Subbarao K. Humans and ferrets with prior H1N1 influenza virus infections do not exhibit evidence of original antigenic sin after infection or vaccination with the 2009 pandemic H1N1 influenza virus. Clin Vaccine Immunol 2014;21(5):737–46.

[29] Greenberg ME, Lai MH, Hartel GF, Wichems CH, Gittleson C, Bennet J, et al. Response to a monovalent 2009 influenza A (H1N1) vaccine. N Engl J Med 2009;361(25):2405–13.

[30] Kung HC, Huang KC, Kao TM, Lee YC, Chang FY, Wang NC, et al. A clinical study to assess the immunogenicity and safety of a monovalent 2009 influenza A (H1N1) vaccine in an area with low-level epidemics of pandemic influenza. Vaccine 2010;28(45):7337–43.

[31] Plennevaux E, Sheldon E, Blatter M, Reeves-Hoché MK, Denis M. Immune response after a single vaccination against 2009 influenza A H1N1 in USA: a preliminary report of two randomised controlled phase 2 trials. Lancet 2010;375(9708):41–8.

[32] Van der Vliet D, Pepin S, Lambert M, Fauchoux N, Donazzolo Y, Dupuy M, et al. Similar immunogenicity of the A/H1N1 2009 pandemic influenza strain when used as a monovalent or a trivalent vaccine. Hum Vaccin 2010;6(10):823–8.