



Effectiveness of the quadrivalent inactivated influenza vaccine in Japan during the 2015–2016 season: A test-negative case-control study comparing the results by real time PCR, virus isolation

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ABSTRACT

Background: We estimated influenza vaccine effectiveness (VE) in 2015–2016 season against medically attended, laboratory-confirmed influenza, when quadrivalent inactivated vaccine (IIV4) was first introduced in Japan, using test-negative case-control design. Influenza A(H1N1)pdm09 cocirculated with B/Yamagata and B/Victoria during the study period in Japan.

Method: We based our case definition on two laboratory tests, real-time reverse transcription polymerase chain reaction (RT-PCR), and virus isolation and compared VEs based on these tests. In addition, VE was evaluated by rapid diagnostic test (RDT). Nasopharyngeal swabs were collected from outpatients who visited clinics with influenza-like illness (ILIs) in Hokkaido, Niigata, Gunma and Nagasaki prefectures.

Results: Among 713 children and adults enrolled in this study, 578 were influenza positive by RT-PCR including, 392 influenza A and 186 influenza B, while 135 were tested negative controls. The adjusted VE by RT-PCR for all ages against any influenza was low protection of 36.0% (95% confidence interval [CI], 3.1% to 58.6%), for influenza A was 30.0% (95% CI: –10.0% to 55.5%), and influenza B was moderate 50.2% (95% CI: 13.3% to 71.4%). Adjusted VE for virus isolation for A(H1N1)pdm09 was 37.1% (95% CI: 1.7% to 59.7%), Yamagata lineage 51.3% (95% CI: 6.4% to 74.7%) and Victoria lineage 21.3% (95% CI: –50.0% to 58.9%). VE was highest and protective in 0–5 years old group against any influenza and influenza A and B/Yamagata, but the protective effect was not observed for other age groups and B/Victoria. RDT demonstrated concordant results with RT-PCR and virus isolation. Sequencing of hemagglutinin gene showed that all A(H1N1)pdm09 belong to clade 6B including 31 strains (88.6%), which belong to clade 6B.1 possessing S162N mutations that may alter antigenicity and affect VE for A(H1N1)pdm09.

Conclusions: IIV4 influenza vaccine during 2015–2016 was effective against A(H1N1)pdm09 and the two lineages of type B. Younger children was more protected than older children and adults by vaccination.

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1. Introduction

Influenza viruses circulate each year causing outbreaks of respiratory illnesses that result in significant morbidity and mortality in

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humans worldwide [1]. Influenza epidemics are estimated to result in about 3 to 5 million severe cases of illness and about 290 000 to 650 000 deaths every year [1]. The majority of deaths associated with influenza are detected among elderly people aged 65 and over [1]. The epidemics are also associated with increased work or school absenteeism and loss of productivity. The World Health Organization (WHO) recommends annual influenza vaccination to groups at risk for influenza complications including pregnant women at any stage of pregnancy, children 6 months to 5 years of age, elderly (aged 65 and over) individuals with underlying chronic medical conditions, and health-care workers [1]. In Japan, the Ministry of Health, Labour and Welfare recommends a universal vaccination strategy which states that all children younger than 13 years of age should receive two doses of vaccine each season and that all others receive one dose.

Annual influenza vaccination is necessary because of the ongoing antigenic drift of influenza viruses and the need to regularly update the vaccine components [2]. Until recently, only trivalent influenza vaccines, which include one strain of influenza B (either B/Yamagata or B/Victoria lineage) in addition to influenza A/H1N1pdm09 and A/H3N2 were used. However, both influenza B lineages have co-circulated during most influenza seasons and often the vaccine strain did not match the predominant circulating strain [3]. The difficulty in predicting which influenza B lineage will dominate in a given season led to the recommendation to include both influenza B lineage strains in the vaccine [4]. During the 2015–2016 season, WHO recommended that quadrivalent vaccines in the Northern Hemisphere should include a B/Brisbane/60/2008 (B/Victoria)-like virus in addition to the other three recommended components for the trivalent vaccine (an A/California/7/2009 (H1N1)pdm09-like virus, an A/Switzerland/9715293/2013 (H3N2)-like virus, and a B/Phuket/3073/2013 (B/Yamagata)-like virus) [5].

Until the 2015–2016 season, the trivalent inactivated subunit-antigen vaccine (TIV) was used in Japan [6], and then from the 2015–2016 season the quadrivalent inactivated vaccine (IIV4) was introduced [7]. Four domestic vaccine manufacturers (Denka Seiken, Co., LTD., Kaketsuken, Kitasato Daiichi Sankyo Vaccine Co., LTD, and Biken Co., LTD.) produce influenza vaccines every year for the use of the Japanese population following the recommendation by the National Institute of Infectious Diseases (NIID) in Japan. In Japan, only domestically produced vaccines are used and the vaccine components selected by NIID basically follow WHO recommendation but are occasionally different from WHO recommended strains, although antigenically similar. It is necessary to measure vaccine effectiveness (VE) of Japanese vaccines to monitor their performance and to compare with those of other countries. The 2015–16 season in Japan has been characterized by dominant influenza A(H1N1)pdm09 activity while, influenza B circulated in a 1:1 ratio between B/Yamagata and B/Victoria, according to the report from the National Institute of Infectious Diseases [7]. Thus, it is a good opportunity to examine VE against two lineages of influenza B that were included in the IIV4 for the first time in Japan. In the current study, we analyzed VE of IIV4 during the season when it was first introduced in Japan, using the test-negative case-control design.

Effectiveness of influenza vaccine by a test-negative case-control design is becoming the most popular observational study method used to estimate influenza vaccine effectiveness and the publication using this method has been reported every year from Australia, Canada, European countries, New Zealand, and the U.S. [8–12]. Real-time polymerase chain reaction (RT-PCR) is a powerful and very sensitive technique for the identification of influenza virus genome used as a gold standard to define “case” in test-negative case-control design to estimate VEs [10,12–18]. However, in Japan, it is common to use rapid diagnostic tests (RDT) in clinical

practice in almost all hospitals and clinics to detect influenza patients, since RDTs are covered by the national health insurance and used as first point of diagnosis and health care, whereas RT-PCR and virus isolation will take several hours and days to get the results [19–21]. Actually, many influenza VE study in Japan used RDTs as a substitute for real-time PCR [6,20,22–24]. Here, we evaluated VE in patients with laboratory-confirmed samples by RT-PCR and virus isolation, supplemented by RDT.

2. Methods

2.1. Study design

Patients with influenza-like illness (body temperature ≥ 37.5 °C, cough, rhinorrhea, malaise), who visited outpatient clinics in Hokkaido, Niigata, Gunma and Nagasaki prefectures between December 2015 and April 2016 were enrolled in this study. The population size is 7843 in Matsumae (Hokkaido prefecture); 807450 in Niigata; 78178 in Shibukawa (Gunma prefecture); 425723 in Nagasaki, and 135546 in Isahaya (Nagasaki prefecture) as of 2016. This study was conducted using test-negative case-control design. Our research was approved by The Niigata University School of Medicine Ethics Committee (No. 1178). Eligible participants or their guardians (usually parents) were informed about the study aims and methods at the outpatient clinics. A written informed consent was obtained from all outpatients who agreed to participate in this study. Nasopharyngeal swabs were obtained and checked by influenza RDT to detect influenza A or B. The following RDTs were used at the discretion of the clinicians: Quick Navi – Flu + RSV (Denka Seiken Co., Ltd. Tokyo, Japan), Quick Navi – Flu (Denka Seiken Co.), Immunoace (TAUNS Laboratories, Inc., Shizuoka, Japan), Drychem Immuno AG1 (Fujifilm Corporation, Tokyo, Japan), Arthonic Flu (Alfresa Holdings Corporation, Osaka, Japan). Information including clinical symptoms, the date of onset, age, sex and influenza vaccination status for the current season were also recorded.

2.2. Vaccine

During the 2015–2016 season, the quadrivalent inactivated subunit-antigen vaccine (IIV4) was used for the first time in Japan. Vaccination was conducted from October until December 2015. The vaccine included the following strains: A/California/7/2009 for A(H1N1)pdm09, A/Switzerland/9715293/2013 for A(H3N2), B/Phuket/3073/2013 for B/Yamagata lineage and B/Texas/2/2013 for B/Victoria lineage [7]. B/Texas/2/2013 for B/Victoria lineage, was an exclusively selected component in Japan but the other three were the same as WHO recommended strains.

In Japan, the recommended vaccination dose is as follows: for children 6 months to 2 years of age two 0.25 ml doses of vaccine 2 to 4 weeks apart, for children 3–12 years two 0.50 ml doses of vaccine 2 to 4 weeks apart, for children over 13 years of age and over only one 0.5 ml dose of vaccine is recommended [6,25]. One vaccine dose was considered as vaccinated in this study even for children under 13 years old to whom twice vaccination is recommended.

2.3. Virus characterization

Viral nucleic acid was extracted from 100 μ l of nasopharyngeal specimens collected during clinic visits using EXTRAGEN II – DNA/RNA extraction kit (Tosoh, Japan). The complementary DNA (cDNA) synthesis was carried out using influenza A and B universal primers (Uni – 11 and Uni – 12) [26]. RT PCR targeting the M gene

was conducted on the original clinical samples to detect influenza A or B [27].

For virus isolation, 100 µl of nasopharyngeal specimen was inoculated onto Madin-Darby canine kidney (MDCK) cells. After inoculation, cultures were monitored for cytopathic effect (CPE) during 3–7 days. Isolates were assayed by cycling probe RT PCR to detect influenza subtypes and lineages: A(H1N1)pdm09, A(H3N2) or B/Yamagata and B/Victoria [28].

The HA genes of A(H1N1)pdm09, influenza B/Yamagata and B/Victoria were amplified by conventional PCR using gene-specific primers (Supplementary Table 1). The PCR products were purified and labelled using BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Carlsbad, USA). Sequencing was run on an ABI Prism 3130xl Genetic Analyzer machine (Applied Bio System, Forest city, USA). Sequencing results were assembled and aligned by using SeqMan Pro software version 12.2.0. The phylogenetic tree analysis was conducted using the Neighbor-Joining method based on the maximum composite likelihood using MEGA 5.2 software [29]. The generated HA sequences were deposited in the GISAID database (www.gisaid.org). Accession numbers are listed in Supplementary Table 2.

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.jvaxc.2019.100011>.

2.4. Test-negative case-control design

VE was estimated using the test-negative case-control design in which a patient who presented with an influenza-like illness and RT PCR-positive for influenza A or B virus was considered as a case, and a patient who presented with an influenza-like illness but was RT PCR-negative was considered as a control. VE was derived as “1- OR (odds ratio) × 100%”, and OR was calculated as $\frac{\text{influenza positive amongst vaccinated} \times \text{influenza negative amongst unvaccinated}}{\text{influenza negative amongst vaccinated} \times \text{influenza positive amongst unvaccinated}}$

In this study, VEs were also estimated for A(H1N1)pdm09, B/Yamagata or B/Victoria based on virus isolation results and additionally for influenza A or B based on RDT.

2.5. Statistical analysis

Univariate analysis was conducted to estimate VE of any influenza infection, influenza A or B by RT-PCR, and A(H1N1)pdm09, B/Yamagata or B/Victoria by virus isolation. Multivariable analysis by logistic regression analysis was carried to adjust for sex (male or female), age (in years), area (Hokkaido, Niigata, Gunma, Nagasaki), and month of onset of illness (January, February, March). One sample collected in December was combined to January and one sample in April combined to March. Furthermore, VE was estimated by age groups (0–5, 6–18 and 19 and over years old) by univariate analysis. Underlying conditions were not included in this analysis because the vast majority of patients were basically otherwise healthy children (86.1%) who visited outpatient clinic due to sudden onset of influenza symptoms. We additionally assessed VEs of RDT for any influenza, influenza A, or B by univariate and multivariable analysis stated as above. All statistical analysis was performed by EZR software version 3.2.2 [30].

3. Results

3.1. Characteristics of the patients

We collected 896 nasopharyngeal swabs from patients who were 6 months to 87 years old with influenza-like illness from Niigata, Hokkaido, Gunma, and Nagasaki in Japan during the 2015/2016 season. A total of 713 patients were enrolled in this study after excluding 183 participants for the following reasons:

unknown vaccination history or missing vaccination status (n = 166), influenza A(H3N2) (n = 2), RDT results were both influenza A and B positive (n = 4), RT PCR was positive for both influenza A and B (n = 8), or virus isolation yielded both influenza A and B viruses (n = 3) (Fig. 1).

Of 713 enrollment patients, 578 were case and 135 were control by RT PCR result. By virus isolation, case and control were divided as 566 and 147, respectively. Baseline characteristics showed that more than half were males (52.7%); vast majority (81.6%) of the enrollees were children and young adults with 268 (37.6%) were 0–5 years old and 346 (48.5%) were 6–18 years old (Table 1). In adult group, 90 (12.6%) were 19–64 years old and 9 (1.3%) were >65 years old. Of all patients enrolled, 357 (50.0%) received influenza vaccine. Once vaccinated and twice vaccinated were 7.2% and 48.8% in children group up to 13 years old. The date of vaccination was not collected from participants in this study. It was not certain whether the cases were enrolled after 14 days of the vaccination. The Ministry of Health, Labour and Welfare in Japan recommends that influenza vaccines should be given until the middle of December prior to influenza season [8]. The first case in our study was enrolled on 29th December 2015, so we assume our participants had enough time to develop immunity after vaccination. More than half of the patients resided in Niigata (n = 407; 57.0%), and then 100 (14.0%) in Hokkaido, 84 (11.8%) in Gunma and 122 (17.1%) in Nagasaki. The 2015–2016 influenza season started in December 2015 and ended in April 2016. The highest number of influenza cases determined by virus isolation was recorded in February (57.5% of the cases) (Fig. 2). One case was registered in December 2015 and another case in April 2016. Number of isolates is based on virus isolation by MDCK cells. Virus isolation using MDCK cells revealed that 361 (50.7%) were A(H1N1)pdm09, 2 A(H3N2), 90 (12.6%) B/Yamagata, 115 (16.1%) B/Victoria, and 147 (20.6%) were negative (Table 1). RDT showed 379 (53.0%) were influenza A, 180 (25.0%) B, and 154 (21.6%) negative.

3.2. VE against laboratory confirmed influenza virus

Assessed by RT PCR, a crude VE during the 2015–2016 season against any influenza was 29.2% (95% CI: –3.0% to 51.5%) (Table 2). Using virus isolation, VE was moderate at 37.1% (95% CI: 9.1% to 56.5%). The adjusted VEs against any influenza by RT PCR and virus

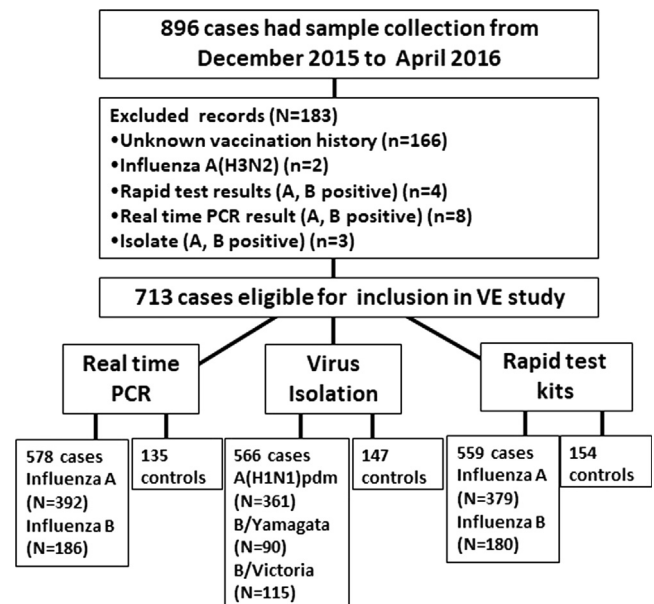


Fig 1. Flow diagram of study participants.

Table 1
Baseline characteristics of the patients.

		Total	Real-time RT-PCR			Virus isolation			
			A (%)	B (%)	Neg (%)	A(H1p09) (%)	B/Yam (%)	B/Vic (%)	Neg (%)
Total		713 (%)	392 (55.0)	186 (26.0)	135 (19.0)	361 (50.7)	90 (12.6)	115 (16.1)	147 (20.6)
Gender	Male	376 (52.7)	205 (54.5)	105 (27.8)	66 (17.5)	183 (48.7)	57 (15.2)	59 (15.7)	77 (20.4)
	Female	337 (47.3)	187 (55.5)	81 (24.0)	69 (20.5)	178 (53.0)	33 (9.7)	56 (16.6)	70 (20.7)
Age(y.o.)	0–5	268 (37.6)	164 (61.2)	44 (16.4)	60 (22.4)	143 (53.3)	14 (5.2)	37 (13.9)	74 (27.6)
	6–18	346 (48.5)	167 (48.3)	123 (35.5)	56 (16.2)	164 (47.4)	59 (17.0)	69 (20.0)	54 (15.6)
	19 and over	99 (13.8)	61 (61.6)	19 (19.2)	19 (19.2)	54 (54.5)	17 (17.2)	9 (9.1)	19 (19.2)
Vaccination status	Vaccinated	357 (50.0)	196 (55.0)	84 (23.5)	77 (21.5)	176 (49.3)	38 (10.6)	56 (15.7)	87 (24.4)
	Unvaccinated	356 (50.0)	196 (55.0)	102 (28.7)	58 (16.3)	185 (52.0)	52 (14.6)	59 (16.6)	60 (16.8)
Place	Niigata	407 (57.0)	262 (64.4)	88 (21.6)	57 (14.0)	249 (61.1)	36 (8.9)	65 (16.0)	57 (14.0)
	Hokkaido	100 (14.0)	34 (34.0)	29 (29.0)	37 (37.0)	28 (28.0)	22 (22.0)	5 (5.0)	45 (45.0)
	Gunma	84 (11.8)	25 (29.8)	37 (44.0)	22 (26.2)	24 (28.6)	15 (17.9)	27 (32.1)	18 (21.4)
	Nagasaki	122 (17.1)	71 (58.2)	32 (26.2)	19 (15.6)	60 (49.2)	17 (13.9)	18 (14.8)	27 (22.1)
Months of clinic visit	December	1 (0.1)	1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	February	410 (57.5)	237 (57.8)	85 (20.7)	88 (21.5)	226 (55.1)	33 (8.0)	61 (14.9)	90 (22.0)
	March	118 (16.4)	21 (17.8)	85 (72.0)	12 (10.2)	16 (13.5)	47 (40.0)	49 (41.5)	6 (5.0)
	April	1 (0.1)	0 (0)	1 (0.1)	0 (0)	0 (0)	1 (0.1)	0 (0)	0 (0)

Abbreviations. A(H1p09): A(H1N1)pdm09, B/Yam: B/Yamagata, B/Vic: B/Victoria, Neg: negative result.

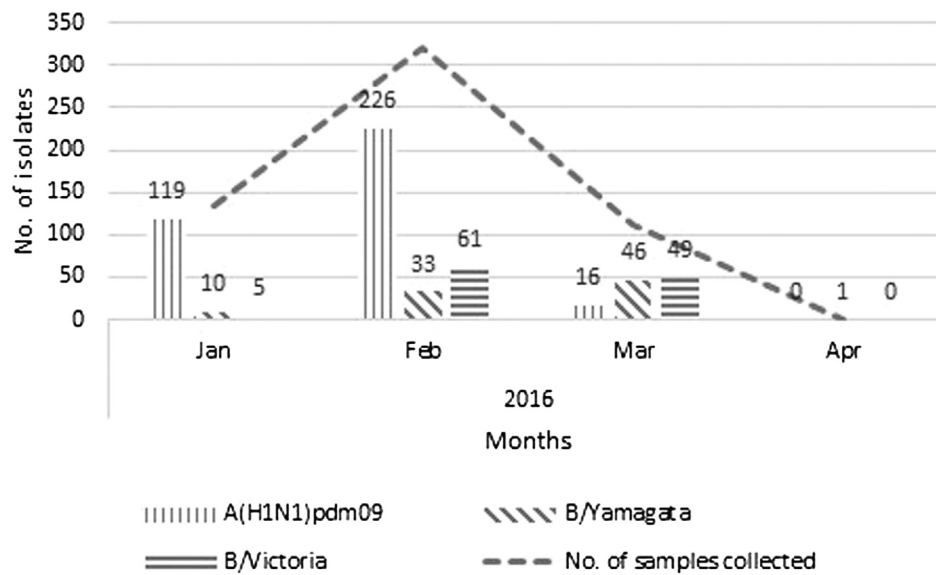


Fig 2. Epidemic curve for the effectiveness of the quadrivalent inactivated influenza vaccine in Japan during the 2015–2016 season.

Table 2
Effectiveness of IIV4 against medically attended influenza during the 2015–2016 season in Japan.

		Case: Vac/non vac	Control: Vac/non vac	Crude (univariate)			Adjusted* (multi-variable)		
				VE (%)	95% CI		VE (%)	95% CI	
All	RT PCR	280/298	77/58	29.2	–3.0	51.5	36.0	3.1	58.6
	Virus isolation	270/296	87/60	37.1	9.1	56.5	40.6	9.9	60.8
A	Typing (RT PCR)	196/197	77/58	24.7	–12.0	49.2	30.0	–10.0	55.5
	Virus isolation: A(H1p09)	176/185	87/60	34.4	3.3	55.5	37.1	1.7	59.7
B	RT PCR	84/102	77/58	38.0	3.0	60.3	50.2	13.3	71.4
	Virus isolation: B/Yam	38/52	87/60	49.6	14.2	70.4	51.3	6.4	74.7
	B/Vic	56/59	87/60	34.5	–7.0	60.0	21.3	–50.0	58.9

VE: vaccine effectiveness, CI: confidence interval, Vac: vaccinated, A(H1p09): A(H1N1)pdm09, B/Yam: B/Yamagata, B/Vic: B/Victoria.

* Adjusted for age, sex, place and month of onset.

isolation were 36.0% (95% CI: 3.1% to 58.6%) and 40.6% (95% CI: 9.9% to 60.8%), respectively.

For influenza A, the adjusted VE was lower at 30.0% (95% CI: –10.0% to 55.5%) by RT PCR compared with virus isolation A (H1N1)pdm09 with VE at 37.1% (95% CI: 1.7% to 59.7%). In this study, influenza A positive by RT PCR is equivalent to A(H1N1)pdm09 by virus isolation because we removed the cases positive for influenza A(H3N2) from the analysis.

Adjusted VE against influenza B using RT PCR was as high as 50.2% (95% CI: 13.3% to 71.4%) and B/Yamagata using virus isolation was similarly high at 51.3% (95% CI: 6.4% to 74.7%). VE against B/Victoria was found low at 21.3% (95% CI: –50.0% to 58.9%).

Analysis of VE by age group revealed that children 0–5 years old consistently showed high VE ranging from 57 to 72% by two tests against any influenza, influenza A and influenza B, including A (H1N1)pdm09, B/Yamagata and B/Victoria (Table 3). In contrast,

Table 3
Vaccine effectiveness by age group in 2015–16 influenza season.

Age group	Case: Vac/non vac	Control: Vac/non vac	Crude (univariate)				
			VE (%)	95% CI			
All	0–5	RT PCR	105/103	44/16	62.9	30.2	80.3
		Virus isolation	93/101	56/18	70.4	46.0	83.8
	6–18	RT PCR	157/133	29/27	–10.0	–95.0	38.0
		Virus isolation	158/134	28/26	–9.0	–96.0	38.8
	19 and over	RT PCR	18/62	4/15	23.7	–170.0	78.5
		Virus isolation	19/61	3/16	–93.0	–839.0	60.3
A	0–5	RT PCR	82/82	44/16	63.6	30.4	81.0
		Virus isolation: A(H1p09)	65/78	56/18	72.3	50.0	85.7
	6–18	RT PCR	101/66	29/27	–42.0	–162.0	22.5
		Virus isolation: A(H1p09)	100/64	28/26	–45.0	–169.0	21.9
	19 and over	RT PCR	13/48	4/15	33.3	–153.0	82.5
		Virus isolation: A(H1p09)	11/43	3/16	–37.0	–629.0	74.4
B	0–5	RT PCR	23/21	44/16	60.2	9.3	82.5
		Virus isolation: B/Yam	7/7	56/18	67.9	–4.0	90.1
		Virus isolation: B/Vic	21/16	56/18	57.8	2.3	81.8
	6–18	RT PCR	56/67	29/27	22.2	–47.0	58.7
		Virus isolation: B/Yam	25/34	28/26	31.7	–43.0	67.5
		Virus isolation: B/Vic	33/36	28/26	14.9	–74.0	58.3
	19 and over	RT PCR	5/14	4/15	–7.0	–413.0	77.6
		Virus isolation: B/Yam	6/11	3/16	–300.0	–2370.0	35.2
		Virus isolation: B/Vic	2/7	3/16	–129.0	–1870.0	73.4

VE: vaccine effectiveness, CI: confidence interval, Vac: vaccinated, A(H1p09): A(H1N1)pdm09, B/Yam: B/Yamagata, B/Vic: B/Victoria.

the other age groups (≥ 6 years old) did not show any good VEs. For influenza A, in 0–5 years old children group, VEs were 63.6% (95% CI: 30.4% to 81.0%) by RT PCR and 72.3% (95% CI: 50% to 85.7%) against A(H1N1)pdm09 by virus isolation. For influenza B, the same age group also showed high VE at 60.2% (95% CI: 9.3% to 82.5%) by RT PCR, 67.9% (95% CI: –4.0% to 90.1%) against B/Yamagata and 57.8% (95% CI: 2.3% to 81.8%) against B/Victoria, but the older age groups (>6 years old) did not show sufficient VEs as in influenza A.

3.3. VE against influenza by RDT

We assessed VE by RDT in the same manner as PCR and virus isolation. Crude VEs against any type, A and B type were 28.1% (95% CI: –3.0% to 49.8%), 25.0% (95% CI: –9.0% to 48.5%) and 34.1% (95% CI: –2.0% to 57.3%) respectively, which are very similar to RT PCR results (Table 4). Adjusted VE against any type of influenza was found as 29.5% (95% CI: –7.0% to 53.6%), against A (H1N1)pdm09 21.2% (95% CI: –24.0% to 49.8%) and 35.1% (95% CI: –14.0% to 63.1%) against B type. The adjusted VEs were similar but lower than RT PCR.

3.4. Genetic characterization

We performed HA gene sequencing of influenza A(H1N1)pdm09 and influenza B to see potential genetic changes that may affect VEs. All of influenza A(H1N1)pdm09 belonged to clade 6B that were further divided into 6B.1 and 6B.2 (Fig. 3). Vast majority of our strains belong to 6B.1 (88.6%) (Supplementary Table 3) and

possessed amino acid mutations of S162N and K163Q (H3 numbering) (Fig. 3) [14,31]. S162N mutation is located in antigenic site Sa that is close to the receptor binding site and adjacent to the clade-defining K163Q mutation [32].

Influenza B strains was genetically divided into B/Yamagata and B/Victoria. Influenza B/Yamagata in this study and vaccine strain B/Phuket/3073/2013 belong to clade 3 (51.0%) (Supplementary Table 3) defined by L172Q substitution (Fig. 4). Influenza B/Victoria and vaccine strain B/Texas/2/2013 (a Japanese influenza vaccine component) belong to clade 1A (49.0%) (Supplementary Table 3) defined mainly by I117V, N129D mutations based amino acid sequence of B/Texas/02/2013 (Fig. 4).

4. Discussion

In this report, we assessed the vaccine effectiveness of influenza in 2015–2016 season, when the quadrivalent vaccine was first introduced in Japan. Overall vaccine effectiveness by laboratory confirmed RT PCR showed mild protection of ~30.0% for any influenza and influenza A but VE was higher as 50.2% against influenza B. Age group analysis showed young children (0–5 years old) demonstrated high protection against any influenza, A and B around 60%, but the older age group (≥ 6 years old) did not have enough protection consistently.

For influenza A, which is A(H1N1)pdm09 in this study, the adjusted influenza vaccine protection was low either by RT PCR 30.0% (95% CI: –10.0% to 55.5%) and virus isolation 37.1% (95% CI: 1.7% to 59.7%). Other Japanese groups reported results of outpatients of 44.0% (95% CI: 13.6% to 63.7%) and 49.0% (95% CI: 42.0% to

Table 4
Vaccine effectiveness of IIV4 by RDT results against medically attended influenza during the 2015–2016 season in Japan.

	Case: Vac/non vac	Control: Vac/non vac	Crude (univariate)		Adjusted [*] (multi-variable)			
			VE (%)	95% CI	VE (%)	95% CI		
Any influenza	270/289	87/67	28.1	–3.0	49.8	29.5	–7.0	53.6
A	187/192	87/67	25	–9.0	48.5	21.2	–24.0	49.8
B	83/97	87/67	34.1	–2.0	57.3	35.1	–14.0	63.1

VE: vaccine effectiveness, CI: confidence interval, Vac: vaccinated.

^{*} Adjusted for age, sex, place and month of onset.

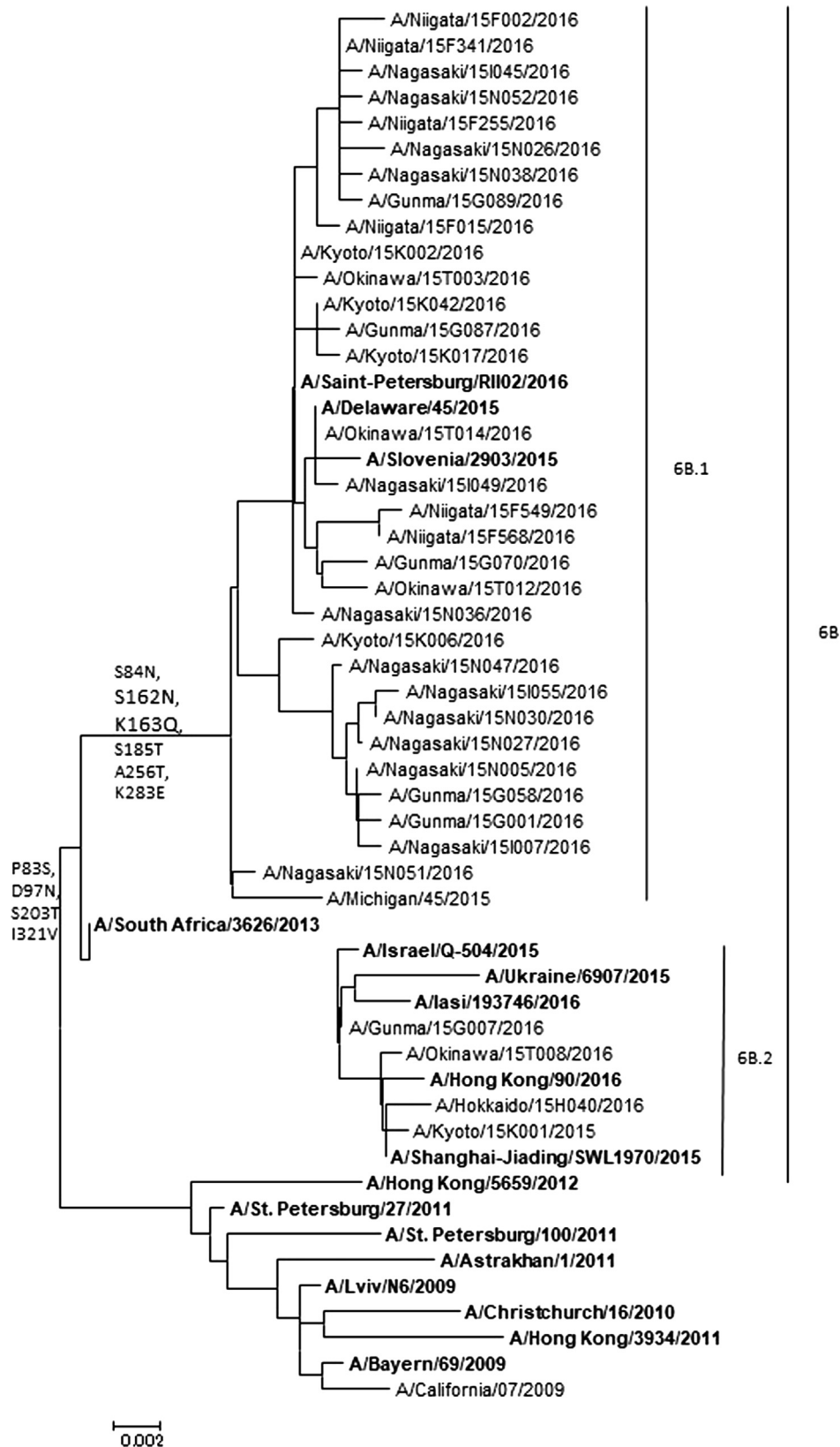


Fig 3. Phylogenetic analysis of the hemagglutinin of influenza A(H1N1)pdm09. Strains were collected in Hokkaido, Niigata, Gunma, Kyoto and Nagasaki, Japan in January to February 2016. Phylogenetic tree was constructed by the neighbor-joining method based on the maximum composite likelihood with MEGA, version 6. Only values greater than 70% are shown. Vaccine component in 2015/2016 season for Northern hemisphere is A/California/07/2009, for Southern Hemisphere in 2017 is A/Michigan/45/2015. Reference sequences and vaccine strains of A(H1N1)pdm09 strains were downloaded from the GISAID EpiFlu Database (www.gisaid.org). The amino acid substitutions relative to the A/California/07/2009 strain are shown at the branch of phylogenetic tree.

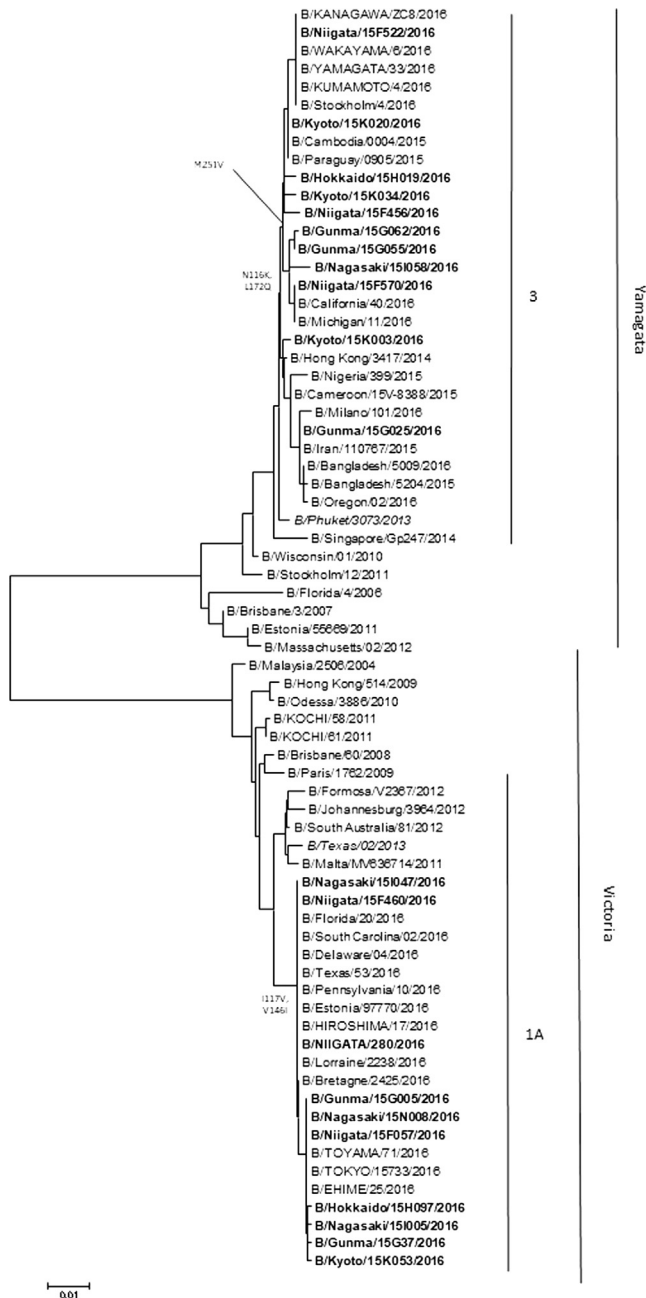


Fig 4. Phylogenetic analysis of the hemagglutinin gene of the two lineages of influenza B virus (B/Yamagata, B/Victoria). Influenza B/Yamagata and B/Victoria strains were collected from Hokkaido, Niigata, Gunma, Kyoto and Nagasaki, Japan in January to February 2016. Phylogenetic tree was constructed by the neighbor-joining method based on the maximum composite likelihood with MEGA, version 6. Only values greater than 70% are shown. Japanese strains collected in this study are shown in bold font. Vaccine components in 2015/2016 season for Northern hemisphere are shown lean font. Reference sequences and vaccine strains of B virus were downloaded from GISAID EpiFlu Database (www.gisaid.org). The amino acid substitutions relative to the B/Phuket/3073/2013 for B/Yamagata lineage and B/Texas/02/2013 strain for B/Victoria lineage are shown in the phylogenetic tree.

54.0%) [20,23] based on RDTs results analyzed by TND. First study recruited only children from 6 months to 15 years old, the second one enrolled adults 16 years old and over, showing the similar VEs between different age groups. We cannot explain the reason for the discordance of VEs against A(H1N1)pdm09 between this study and another Japanese studies. However, a possible reason for our low VE against A(H1N1)pdm09 can be explained by the antigenic change of circulating clade 6B.1 viruses. Influenza A

(H1N1)pdm09 virus belonging to Clade 6B.1 possesses S162N mutation that confers a potential gain of glycosylation [14,23]. WHO reported that 6B.1 and 6B.2 viruses poorly reacted with post-vaccination adult human serum pools against A/California/7/2009 [32]. Reports from the United States also supported that the binding of human sera from the subjects infected with A(H1N1)pdm09 viruses to the HA proteins between recently circulating strains and vaccine strain was not identical, strongly suggesting antigenic differences in the HA protein between the two strains [31,33]. Due to the antigenic changes observed with 6B.1 and 6B.2 viruses, WHO changed a vaccine component for Southern Hemisphere influenza vaccine in 2017 from A/California/7/2009 to A/Michigan/45/2015. Thus, the low VE against A(H1N1) pdm09 in this study may be the result from antigenic mismatches between the former vaccine component and the circulating strains [23]. Indeed, reports from the US during the same season showed low VEs of 49.0% (95% CI: 37.0% to 59.0%) for IIV4 [34], 43.0% (95% CI: 25.0% to 57.0%) for TIV in Canada [35] and 32.9% (95% CI: 15.5% to 46.7%) in Europe [36], which are explained by changes in the circulating strain compared to the vaccine strain.

Against overall influenza B and B/Yamagata, we obtained moderate effectiveness of 50.2% (95% CI: 13.3% to 71.4%) and 51.3% (95% CI: 6.4% to 74.7%) respectively, but B/Victoria showed low effectiveness of 21.3% (95% CI: -50.0% to 58.9%). Another Japanese group reported low VE of 33.8% (95% CI: 25% to 64.8%) but they did not specify the lineages of influenza B [23]. Researchers from the United States, reported VE of 66.0% (95% CI: 51.0% to 76.0%) against B/Yamagata, and 57.0% (95% CI: 36.0% to 71.0%) for influenza B/Victoria from patients who received IIV4 during the 2015–2016 season [34]. A study in Canada reported VE of 54.0% (95% CI: 32.0% to 68.0%) against B/Victoria [35] despite lineage mismatch with vaccine strain for B/Yamagata. The L172Q mutation in HA of B/Yamagata that was found in our study may cause potential antigenic change when compared to the vaccine strain B/Phuket/3073/2013, but we did not observe any reduction of VEs for B/Yamagata lineage. The lower VEs for the B/Victoria in this study may be explained by our unpublished data that antibody titers against B/Victoria were lower than B/Yamagata after the IIV4 vaccination during the 2015/2016 season.

WHO recommended to reformulate the TIV into IIV4 by including both influenza B lineages (Yamagata and Victoria), since TIV may provide only limited cross-protection against strains in the other lineage [37]. Predicting which B virus lineage will predominate during a given season has been a challenge over the years. A recent modeling analysis showed that during the 10 seasons from 2001 to 2002 through 2010–2011, the predominant circulating influenza B virus lineage matched to the trivalent vaccine in only five seasons [3]. Thus, inclusion of two B virus strains in seasonal influenza vaccines was expected to improve the protection against circulating seasonal B virus strains. Indeed, we saw a good protection against overall influenza B during the 2015–2016 season in this study, but follow up investigation is needed [20,22,24].

In our study, vaccine effectiveness against any influenza and influenza A was high in small children aged 0–5 years old. Similar better protection in small children was reported from various Japanese group including our group in the past [6,24]. In 2011–2012 season in Japan, the trivalent influenza vaccine demonstrated vaccine effectiveness in 1–2 and 3–5 years old groups: 55.0% (95% CI: 40.0% to 66.0%), 32% (95% CI: 17.0% to 45.0%) respectively [24]. Then, during 2013–2014 and 2014–2015 seasons in Japan, TIV demonstrated vaccine effectiveness of 38.0–45.0% in children 6 months –15 years old [20]. For IIV4, a concordance of VE estimates between our study and previous study in Europe was found for 1–5 years old group and the VE values decreased in older age groups [12]. Another group from Japan reported VE of 39.0% (95% CI: 28.0% to 46.0%) against influenza infection overall and 40.0%

(95% CI: 18.0% to 56.0%) in 1–2 years old group and 55.0% (95% CI: 41.0% to 65.0%) in 3–5 years old [22]. These results are similar with our findings. One possible reason is that when small children received influenza vaccine, usually their family members also received influenza vaccine together, thereby indirect protection to their children was provided [6]. In contrast, the VE of school age children 6–18 years old in our study was lower compared to young children. This decreasing trend of VEs over the age groups can be explained by decreased protection with repeated influenza vaccination. Reports in the 2015–2016 season were focused on decreased VEs against influenza among repeated recipients of inactivated vaccines compared to the current season only recipients, especially A(H1N1)pdm09 [34–36]. At the same time these reports showed decreased protection over the age groups. This may be explained by the “original antigenic sin”, where original childhood priming exposures may have induced selective memory responses and decreased antibody production after repeated vaccination [38,39]. In our study, past vaccination history of the participants is unknown. Our past report showed that vaccine coverage in a city in Japan among children under 12 years old were 60–70% during 2011–2012 and 2012–2013 seasons [24]. The reason for high vaccine coverage of children is that local governments in Japan support influenza vaccination by subsidizing its cost. Thus, it was likely that school age children and older adults have received vaccines multiple times in the past and showed reduced VEs.

RDT showed compatible VEs in this study when compared to RT PCR method, which is regarded as a gold standard for detecting influenza viruses. Many of past reports from Japan were based on RDT, where sensitivity and specificity of RDT are regarded as relatively high in Japanese settings, 62.3–100%, and 94.0–100%, respectively [20–22,24]. In our study, high sensitivity (~90%) and comparable specificity (70–90%) of RDTs were demonstrated against RT PCR and virus isolation (Supplementary Table 4). Previous reports showed that lower specificity of RDT tends to underestimate VE [40,41]. Indeed, our VEs of RDT were slightly lower than those of RT PCR, which is compatible with the past findings of underestimated VE values. Influenza RDTs are generally regarded as having low sensitivity, making it less reliable. A recent meta-analysis from USA showed that pooled sensitivities for detecting influenza A from Bayesian bivariate random-effects models were 54.4% (95% CI: 48.9% to 59.8%) and that for influenza B were 53.2% (95% CI: 41.7% to 64.4%) [42]. The reason for the discrepancy of our results and the other reports can be explained by high virus titer in Japanese patients. Many of patients visit outpatient clinics just after their onset within one or two days when they shed virus at their peak [43]. This situation resulted in higher sensitivity and good much with RT PCR results.

One of the limitations of this study is the small sample size of controls. We excluded 166 patients with unknown vaccination history to minimize the possibility of underestimating VE, but this led to the decrease in sample size. In our study, the ratio of cases to controls was low at 1:0.2. The low number of controls as well as the small sample size of influenza B and some age groups resulted in lower precision and wider confidence intervals. Second, test-negative case control study has potential biases. One of the scenarios is the overestimation of VE if vaccinated patients had mild symptoms and did not seek medical care [44]. Another is the underestimation of VE due to larger number of vaccinated persons who have not contracted influenza illness and stay home compared to lesser number of those unvaccinated and healthy. We have to take note on these potential biases when estimating VE using test negative case control design. Third, it is reported that the lower specificity of the test leads to the underestimation of VE [40]. However, our virus isolation showed higher VEs compared to RT PCR despite the low specificity just like RDT. We cannot explain why it happened to virus isolation but not to RDT which

in turn had tendency to underestimate VE. Another limitation is the unclear evidence of vaccination date. In this study, the date of vaccination was not collected due to the timing of influenza vaccination program in Japan, which was finished in the middle of December while the first sample was collected in the end of December.

In conclusion, the current study demonstrated low effectiveness against any influenza infections, A(H1N1)pdm09, and moderate effectiveness for B/Yamagata for all age. In young children, we obtained high VE, but low VE was observed for school age children and adults. A strong points of this study is that we were able to evaluate VE using three different tests, RT PCR as gold standard and compared VEs with other tests, virus isolation and RDT. In addition, we evaluated VEs by influenza B lineages. Future work is needed to obtain a more precise VE estimate and to clarify VE by age groups and lineages of influenza B.

Declaration of interests

The authors declare that there are no known conflicts of interest.

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References

- [1] World Health Organization. Influenza (Seasonal), Details, Fact Sheets, News, World Health Organization [<http://who.int/mediacentre/factsheets/fs211/en/> accessed on 25 Apr 2018].
- [2] Carrat F, Flahault A. Influenza vaccine: the challenge of antigenic drift. *Vaccine* 2007;25(39–40):6852–62. <https://doi.org/10.1016/j.vaccine.2007.07.027>. PubMed PMID: 17719149.
- [3] Ambrose CS, Levin MJ. The rationale for quadrivalent influenza vaccines. *Hum Vaccin Immunother* 2012;8(1):81–8. <https://doi.org/10.4161/hv.8.1.17623>. PubMed PMID: 22252006.
- [4] Thomas R, Frieden M, MPH, Director, Harold W. Jaffe M, MA, Associate Director for Science Joanne Cono, MD, ScM, Acting Director, Office of Science Quality Chesley L. Richards, MD, MPH, Deputy Director, Office of Public Health Scientific Services (proposed), Pamela S. Diaz M, Acting Director, Center for Surveillance, Epidemiology, and Laboratory Services (proposed). Prevention and control of seasonal influenza with vaccines. Recommendation of the advisory committee on immunization practices- United States 2013–2013.
- [5] World Health Organization. Recommended composition of influenza virus vaccines for use in the 2015–2016 northern hemisphere influenza season. *Weekly Epidemiological Record*. 2015;90(11):97–108.
- [6] Shinjoh M, Sugaya N, Yamaguchi Y, Tomidokoro Y, Sekiguchi S, Mitamura K, et al. Effectiveness of trivalent inactivated influenza vaccine in children estimated by a test-negative case-control design study based on influenza rapid diagnostic test results. *PLoS One* 2015;10(8):e0136539. <https://doi.org/10.1371/journal.pone.0136539>. PubMed PMID: 26317334.
- [7] National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division Ministry of Health Labour and Welfare. Influenza 2015/16 season, Japan. *Infectious Agents Surveillance Report (IASR)*. 2016;37(11):211–3.

- [8] Skowronski DM, Masaro C, Kwindt TL, Mak A, Petric M, Li Y, et al. Estimating vaccine effectiveness against laboratory-confirmed influenza using a sentinel physician network: Results from the 2005–2006 season of dual A and B vaccine mismatch in Canada. *Vaccine* 2007;25(15):2842–51. <https://doi.org/10.1016/j.vaccine.2006.10.002>. PubMed PMID: 17081662.
- [9] Turner N, Pierse N, Huang QS, Radke S, Bissielo A, Thompson MG, et al. Interim estimates of the effectiveness of seasonal trivalent inactivated influenza vaccine in preventing influenza hospitalisations and primary care visits in Auckland, New Zealand, in 2014. *Eurosurveillance* 2014;19(42):19–24. PubMed Central PMCID: PMC25358042.
- [10] McLean HQ, Thompson MG, Sundaram ME, Kieke BA, Gaglani M, Murthy K, et al. Influenza vaccine effectiveness in the United States during 2012–2013: variable protection by age and virus type. *J Infect Dis* 2015;211(10):1529–40. <https://doi.org/10.1093/infdis/jiu647>. PubMed Central PMCID: PMCPC4407759.
- [11] K Bragstad HDE, T K Fischer, M Voldstedlund, S Gubbels, B Andersen, K Mølbak, T G Krause. Low vaccine effectiveness against influenza A(H3N2) virus among elderly people in Denmark in 2012–13 – a rapid epidemiological and virological assessment. *PubMed Central PMCID: PMC23449183*.
- [12] Valenciano M KE. Early estimates of seasonal influenza vaccine effectiveness in Europe results from the I-MOVE multicentre case-control study, 2012–13. 2013. PubMed Central PMCID: PMC23449183.
- [13] World Health Organization. Global Epidemiological Surveillance Standards for Influenza [http://www.who.int/influenza/resources/documents/WHO_Epidemiological_Influenza_Surveillance_Standards_2014.pdf accessed on 25 Apr 2018]. 2014.
- [14] Chambers C, Skowronski DM, Sabaiduc S, Winter AL, Dickinson JA, De Serres G, et al. Interim estimates of 2015/16 vaccine effectiveness against influenza A (H1N1)pdm09, Canada, February 2016. *Euro Surveill* 2016;21(11):30168. <https://doi.org/10.2807/1560-7917.ES.2016.21.11.30168>. PubMed PMID: 27020673.
- [15] Zhang Y, Wu P, Feng L, Yang P, Pan Y, Feng S, et al. Influenza vaccine effectiveness against influenza-associated hospitalization in 2015/16 season, Beijing, China. *Vaccine* 2017;35(23):3129–34. <https://doi.org/10.1016/j.vaccine.2017.03.084>. PubMed PMID: 28456530.
- [16] Cowling BJ, Kwan MY, Wong JS, Feng S, Leung CW, Chan EL, et al. Interim estimates of the effectiveness of influenza vaccination against influenza-associated hospitalization in children in Hong Kong, 2015–16. *Influenza Other Respir Viruses* 2017;11(1):61–5. <https://doi.org/10.1111/irv.12399>. PubMed PMID: 27313064.
- [17] Puig-Barbera J, Guglieri-Lopez B, Tortajada-Girbes M, Lopez-Labrador FX, Carballido-Fernandez M, Mollar-Maseres J, et al. Low influenza vaccine effectiveness and the effect of previous vaccination in preventing admission with A(H1N1)pdm09 or B/Victoria-Lineage in patients 60 years old or older during the 2015/2016 influenza season. *Vaccine* 2017;35(52):7331–8. <https://doi.org/10.1016/j.vaccine.2017.10.100>. Epub 2017/11/13 PubMed PMID: 29128380.
- [18] Valdin HL, Begue RE. Influenza vaccines effectiveness 2013–14 through 2015–16, a test-negative study in children. *Vaccine* 2017;35(33):4088–93. <https://doi.org/10.1016/j.vaccine.2017.06.050>. Epub 2017/07/04 PubMed PMID: 28669621.
- [19] Caroline Chartrand MMG, Leeflang, Jessica Minion, Timothy Brewer, Madhukar Pal. Accuracy of rapid influenza diagnostic kit. a meta-analysis. *Annals of internal medicine*. 2012.
- [20] Sugaya N, Shinjoh M, Nakata Y, Tsunematsu K, Yamaguchi Y, Komiyama O, et al. Three-season effectiveness of inactivated influenza vaccine in preventing influenza illness and hospitalization in children in Japan, 2013–2016. *Vaccine* 2018;36(8):1063–71. <https://doi.org/10.1016/j.vaccine.2018.01.024>. PubMed PMID: 29361343.
- [21] Suzuki M, Minh le N, Yoshimine H, Inoue K, Yoshida LM, Morimoto K, et al. Vaccine effectiveness against medically attended laboratory-confirmed influenza in Japan, 2011–2012 Season. *PLoS One* 2014;9(2). <https://doi.org/10.1371/journal.pone.0088813>. Epub 2014/02/20 PubMed PMID: 24551167; PubMed Central PMCID: PMCPC3923823.
- [22] Sugaya N, Shinjoh M, Kawakami C, Yamaguchi Y, Yoshida M, Baba H, et al. Trivalent inactivated influenza vaccine effective against influenza A(H3N2) variant viruses in children during the 2014/15 season, Japan. *Euro Surveill* 2016;21(42). <https://doi.org/10.2807/1560-7917.ES.2016.21.42.30377>. PubMed PMID: 27784529.
- [23] Seki Y, Onose A, Sugaya N. Influenza vaccine effectiveness in adults based on the rapid influenza diagnostic test results, during the 2015/16 season. *J Infect Chemother* 2017;23(9):615–20. <https://doi.org/10.1016/j.jiac.2017.06.004>. PubMed PMID: 28668446.
- [24] Suzuki T, Ono Y, Maeda H, Tsujimoto Y, Shobugawa Y, Dapac C, et al. Effectiveness of Trivalent Influenza Vaccine among Children in Two Consecutive Seasons in a Community in Japan. *Tohoku J Exper Med* 2014;232(2):97–104. <https://doi.org/10.1620/tjem.232.97>. PubMed PMID: 24531035.
- [25] Ministry of Health LaW. FY2003 Influenza Q&A [https://www.mhlw.go.jp/bunya/kenkou/kekaku-kansenshou01/qa.html#q26/ cited December 5 2018]. Japanese.
- [26] Hoffmann E, Stech J, Guan Y, Webster RG, Perez DR. Universal primer set for the full-length amplification of all influenza A viruses. *Arch Virol* 2001;146(12):2275–89. <https://doi.org/10.1007/s007050170002>.
- [27] Kondo H, Shobugawa Y, Hibino A, Yagami R, Dapac C, Okazaki M, et al. Influenza Virus Shedding in Laninamivir-Treated Children upon Returning to School. *Tohoku J Exper Med* 2016;238(2):113–21. <https://doi.org/10.1620/tjem.238.113>. Epub 2016/01/26 PubMed PMID: 26806610.
- [28] Suzuki Y, Saito R, Zaraket H, Dapac C, Caperig-Dapac I, Suzuki H. Rapid and specific detection of amantadine-resistant influenza A viruses with a Ser31Asn mutation by the cycling probe method. *J Clin Microbiol* 2010;48(1):57–63. <https://doi.org/10.1128/JCM.00698-09>. PubMed PMID: 19889895.
- [29] Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*. 2011;28(10):2731–9. <https://doi.org/10.1093/molbev/msr121>. PubMed PMID: 21546353.
- [30] Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 2013;48(3):452–8. <https://doi.org/10.1038/bmt.2012.244>. PubMed PMID: 23208313.
- [31] Clark AM, DeDiego ML, Anderson CS, Wang J, Yang H, Nogales A, et al. Antigenicity of the 2015–2016 seasonal H1N1 human influenza virus HA and NA proteins. *PLoS One* 2017;12(11):e0188267. <https://doi.org/10.1371/journal.pone.0188267>. PubMed PMID: 29145498.
- [32] Gubareva LV, Besselaar TG, Daniels RS, Fry A, Gregory V, Huang W, et al. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2015–2016. *Antiviral Res* 2017;146:12–20. <https://doi.org/10.1016/j.antiviral.2017.08.004>. Epub 2017/08/15 PubMed PMID: 28802866; PubMed Central PMCID: PMCPC5667636.
- [33] Linderman SL, Chambers BS, Zost SJ, Parkhouse K, Li Y, Herrmann C, et al. Potential antigenic explanation for atypical H1N1 infections among middle-aged adults during the 2013–2014 influenza season. *Proc Natl Acad Sci USA* 2014;111(44):15798–803. <https://doi.org/10.1073/pnas.1409171111>. Epub 2014/10/22 PubMed PMID: 25331901; PubMed Central PMCID: PMCPC4226110.
- [34] Jackson ML, Chung JR, Jackson LA, Phillips CH, Benoit J, Monto AS, et al. Influenza Vaccine Effectiveness in the United States during the 2015–2016 Season. *N Engl J Med* 2017;377(6):534–43. <https://doi.org/10.1056/NEJMoai700153>. Epub 2017/08/10 PubMed PMID: 28792867; PubMed Central PMCID: PMCPC5727917.
- [35] 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(12):1487–500. <https://doi.org/10.1093/infdis/jix526>. Epub 2017/10/14 PubMed PMID: 29029166; PubMed Central PMCID: PMCPC5853508.
- [36] Kissling E, Valenciano M, Pozo F, Vilcu AM, Reuss A, Rizzo C, et al. 2015/16 I-MOVE/I-MOVE+ multicentre case-control study in Europe: Moderate vaccine effectiveness estimates against influenza A(H1N1)pdm09 and low estimates against lineage-mismatched influenza B among children. *Influenza Other Respir Viruses* 2018;12(4):423–37. <https://doi.org/10.1111/irv.12520>. Epub 2017/11/11 PubMed PMID: 29125681; PubMed Central PMCID: PMCPC6005601.
- [37] World Health Organization. Recommended composition of influenza virus vaccines for use in the 2016–2017 northern hemisphere influenza season [http://www.who.int/influenza/vaccines/virus/recommendations/2016_17_north/en/ accessed on 25 Apr 2018]. 2016.
- [38] Sanyal M, Holmes TH, Maecker H, Albrecht RA, Dekker CL, He XS, et al. Diminished B-cell response after repeat influenza vaccination. *J Infect Dis* 2018. <https://doi.org/10.1093/infdis/jiy685>. Epub 2018/11/30 PubMed PMID: 30496437.
- [39] Henry C, Palm AE, Krammer F, Wilson PC. From original antigenic sin to the universal influenza virus vaccine. *Trends Immunol* 2018;39(1):70–9. <https://doi.org/10.1016/j.it.2017.08.003>. Epub 2017/09/05 PubMed PMID: 28867526; PubMed Central PMCID: PMCPC5748348.
- [40] Ferdinands JM, Shay DK. Magnitude of potential biases in a simulated case-control study of the effectiveness of influenza vaccination. *Clin Infect Dis* 2012;54(1):25–32. <https://doi.org/10.1093/cid/cir750>. Epub 2011/11/19 PubMed PMID: 22095567.
- [41] Orenstein EW, De Serres G, Haber MJ, Shay DK, Bridges CB, Gargiullo P, et al. Methodologic issues regarding the use of three observational study designs to assess influenza vaccine effectiveness. *Int J Epidemiol* 2007;36(3):623–31. <https://doi.org/10.1093/ije/dym021>. PubMed PMID: 17403908.
- [42] Merckx J, Wali R, Schiller I, Caya C, Gore GC, Chartrand C, et al. Diagnostic accuracy of novel and traditional rapid tests for influenza infection compared with reverse transcriptase polymerase chain reaction: a systematic review and meta-analysis. *Ann Intern Med* 2017;167(6):394–409. <https://doi.org/10.7326/M17-0848>. Epub 2017/09/05 PubMed PMID: 28869986.
- [43] Zaraket H, Saito R. Japanese surveillance systems and treatment for influenza. *Curr Treat Options Infect Dis* 2016;8(4):311–28. <https://doi.org/10.1007/s40506-016-0085-5>. Epub 2016/12/31 PubMed PMID: 28035195; PubMed Central PMCID: PMCPC5155020.
- [44] Shi M, An Q, Ainslie KEC, Haber M, Orenstein WA. A comparison of the test-negative and the traditional case-control study designs for estimation of influenza vaccine effectiveness under nonrandom vaccination. *BMC Infect Dis* 2017;17(1):757. <https://doi.org/10.1186/s12879-017-2838-2>. Epub 2017/12/09 PubMed PMID: 29216845; PubMed Central PMCID: PMCPC5721721.