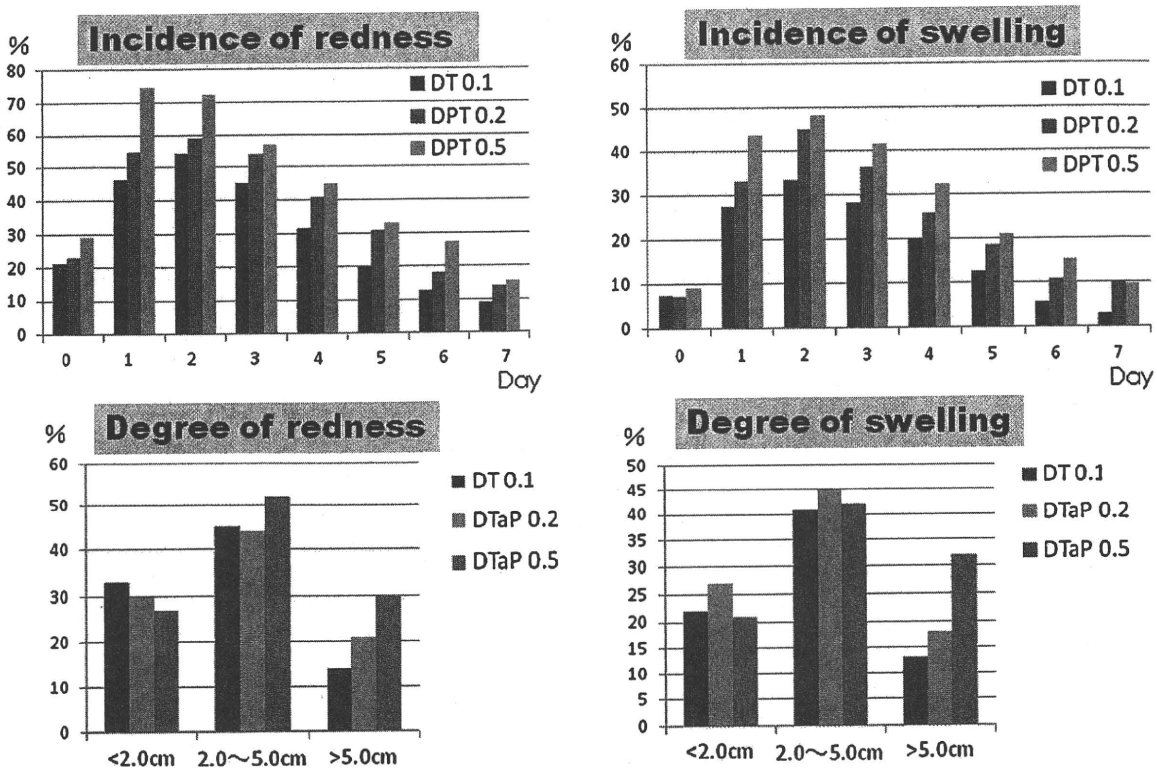

Fig. 4. Onset of local redness and swelling, and the severity of adverse events.

three groups, but 0.5 ml of DTaP had a tendency to induce a serious local reaction (redness and swelling) >5.0 cm.

3.4. Immunogenicity

Study group 1, in whom paired serum samples were examined, consisted of 266 subjects with serological examination, 29 with 0.1 ml of DT, 119 with 0.2 ml of DTaP, and 118 with 0.5 ml of DTaP. The sero-positivity of antibodies for diphtheria toxoid >0.1 was 60.9% (162/266), 90.6% (241/266) for tetanus toxoid >0.01, 54.13% (144/266) for PT >10, and 82.33% (219/266) for FHA > 10 EIA units. Antibodies against PT were markedly reduced at the age of 11–12 years.

The results of sero-positivity and GMT are shown in Table 4. The sero-positivity of PT and FHA and their GMT were the same before and after immunization in the DT 0.1 ml group. After immunization, the sero-positivity against PT increased from 52.1 to 95% in the DTaP 0.2 ml group and from 55.1 to 95.8% in the DTaP 0.5 ml group. The GMT of PT antibodies after immunization with 0.2 ml of DTaP was 89.05 (95% CI: 70.54–112.41), and there was no significant difference after immunization with 0.5 ml of DTaP, being 102.74 (95% CI: 82.91–127.32). Sero-positivity against FHA increased from 85.7 to 100% in the DTaP 0.2 ml group and from 78.8 to 98.3% in the DTaP 0.5 ml group. The GMT of antibodies against FHA was 252.82 (95% CI: 214.29–298.27) after immunization with 0.2 ml of DTaP and 302.06 (95% CI: 254.2–358.93) after immunization with 0.5 ml of DTaP, without a significant difference. Sero-positivity against diphtheria toxoid was 55.9–66.4% before immunization and increased to 100% in all three groups. The GMT of antibodies against diphtheria toxoid was 40.14 (95% CI: 28.28–56.96), 45.17 (95% CI: 35.59–57.32), and 46.78 (95% CI: 35.73–61.24) in the DT 0.1 ml, DTaP 0.2 ml, and DTaP 0.5 ml groups, respectively. As for the antibodies against tetanus toxoid, 86.2–94.1% sero-positivity before immunization increased to 100%. The GMT of antibodies against tetanus toxoid after vaccination with 0.2 ml of DTaP was 18.02 (95% CI: 14.90–21.80), similar to the 20.96 (95% CI: 13.37–32.84) after immunization with 0.1 ml of DT. However, the GMT of antibodies against tetanus toxoid was 27.12 (95% CI: 22.79–32.27) after immunization with 0.5 ml of DTaP, higher than those in DT 0.1 ml and DTaP 0.2 ml groups.

3.5. Difference in immunogenicity of different brands

There was no significant difference in immunogenicity against PT and FHA after immunization with 0.2 or 0.5 ml of DTaP. Risk ratios of a local reaction to 0.5 ml of DTaP compared to 0.1 ml of DT were higher than that to 0.2 ml of DTaP. GMTs after immunization with different brands of DTaP are shown in Fig. 5. A volume of 0.2 ml of DTaP contained 1.2–9.4 μg of PT, 9.4–20.6 μg of FHA, 6–6.6 Lf of diphtheria toxoid, and 1.0 Lf of tetanus toxoid. A volume of 0.1 ml of DT contains similar amounts of tetanus and diphtheria toxoid antigens in different brands and compared with 0.2 ml of each DTaP brand. 29 were immunized with 0.1 ml DT, 26 with 0.2 ml of Takeda DTaP, 26 with Biken, 19 with Kaketsu, 19 with Kitasato, and 29 with Denka. There was no significant difference in GMTs of antibodies against diphtheria toxoid after immunization with the five different brands in comparison with that induced after immunization with 0.1 ml of DT. The GMT against tetanus toxoid after immunization with Kitasato was higher than that after 0.1 ml of DT. As for the pertussis antigens, the GMT of PT antibodies after immunization with Takeda or Denka vaccine was lower than those induced after the other brands. These two brands contained lower amounts of PT antigen. The GMT against FHA after immunization with Denka was slightly lower than the others, not reflecting the concentration of vaccine material.

4. Discussion

Pertussis is an infectious disease affecting young infants and children, leading to severe illness in very young infants,

Table 4
Immunogenicity of DT and DTaP.

	DT 0.1 ml		DTaP 0.2 ml		DTaP 0.5 ml	
	Sero+ rate GMT pre (95% CI)	Sero+ rate GMT post (95% CI)	Sero+ rate GMT pre (95% CI)	Sero+ rate GMT post (95% CI)	Sero+ rate GMT pre (95% CI)	Sero+ rate GMT post (95% CI)
Anti-PT	58.6% 10.8 (6.38–18.29)	58.6% 13.93 (8.98–21.61)	52.1% 12.11 (9.21–15.94)	95% 89.05 (70.54–112.41)	55.1% 10.88 (8.27–14.32)	95.8% 102.74 (82.91–127.32)
Anti-FHA	82.8% 24.92 (16.34–38.00	86.2% 31.2 (22.43–43.42)	85.7% 33.73 (27.32–41.64	100% 252.82 (214.29–298.27)	78.8% 25.83 (20.67–32.28)	98.3% 302.06 (254.2–358.93)
Anti-D	58.6% 0.23 (0.11–0.471)	100% 40.14 (28.28–56.96)	66.4% 0.22 (017–0.30)	100% 45.17 (35.59–57.32)	55.9% 0.16 (0.12–0.24)	100% 46.78 (35.73–61.24)
Anti-T	86.2% 0.47 (0.28–0.81)	100% 20.96 (13.37–32.84)	94.1% 0.87 (0.70–1.09)	100% 18.02 (14.90–21.80)	88.1% 0.59 (0.44–0.79)	100% 27.12 (22.79–32.27)

causing whoop, staccato, apnea, and choking with sputa. To prevent the disease, acellular pertussis vaccines have been used in many developed countries. However, the acellular vaccine did not confer a long-lasting antibody response after vaccination and so in the late 1990s several pertussis outbreaks occurred in young adults [10–16]. The diagnosis of pertussis in adults was difficult because they only demonstrated mild atypical symptoms, showing a prolonged cough without whooping [24–26]. The adult patients showing a prolonged cough were not suspected to have pertussis because general physicians believed that pertussis was a disease only affecting children. They were, therefore, undiagnosed, and the number of patients with pertussis was underreported. In addition, they were not treated and transmitted pertussis to young infants before DTaP immunization [27]. The adult pertussis vaccine trial was conducted in 2781 subjects consisting of 1391 received the acellular pertussis vaccine and 1390 received the control vaccine. Ten patients of pertussis were diagnosed by culture, PCR, or serological responses and nine were in the control group and one in acellular pertussis vaccine group. An incidence of 370–450 cases per 100,000 person-years was noted in the control group aged 15–65 years and the acellular pertussis vaccine was protective in the same age group [28]. These adult patients with pertussis were considered to be an infectious source for transmission to young infants in household contact. Through such household contacts, even vaccinated children who had been completely immunized showed typical pertussis, and the most likely source of infant

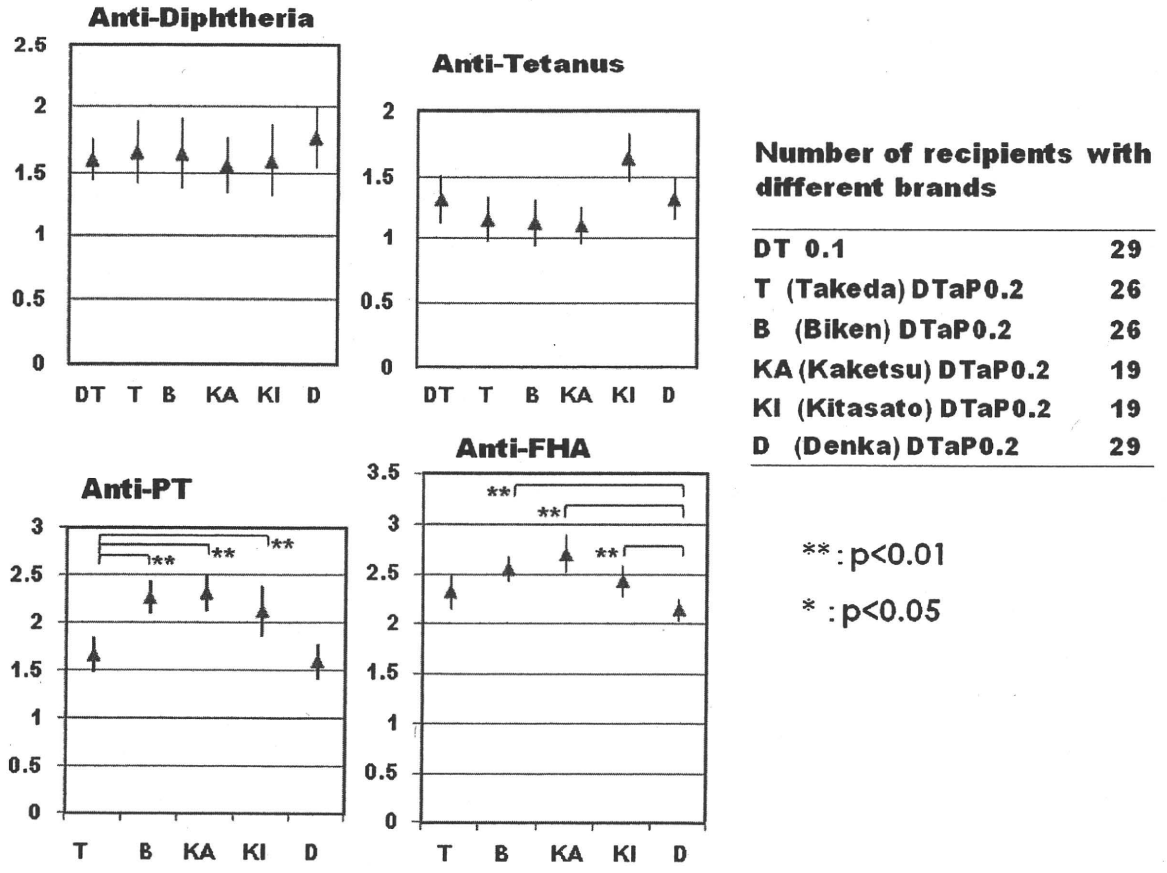

Fig. 5. GMTs of anti-D, T PT, and FHA antibodies after immunization with different brands of DTaP at 0.2 ml.

infection was reported, being a sibling (41%), mother (38%), and father (17%). To control pertussis, Tdap was developed and recommended as the booster in teenagers and young adults [15]. It is necessary to maintain a high level of immunity in all generations [29,30]. Thus, Tdap was newly recommended for all generations from 19 to 64 years as well as teenagers [17,18].

DTaP was first developed in Japan and has been used since 1981 [4]. Some pertussis patients were reported sporadically in Japan, and a survey of 89 households showed that the source of infection was an adult in approximately 11% and the secondary attack rate was 10%, confirmed by serological responses with asymptomatic infection [31]. The estimated efficacy of DTaP was 84% (95% CI: 71–91%) in children aged 2–8 years. Since vaccine-induced immunity waned 6–10 years after immunization, immunization with vaccines including pertussis components was proposed for both children and adults [32]. Adult patients with pertussis have gone undiagnosed and, therefore, the disease burden of pertussis has been neglected. In 2007–08, there were several outbreaks in universities, schools, and other facilities, and the number of reported cases of pertussis increased. Most of the patients were over 15 years of age and, the number of patients aged less than 1 year increased.

To control pertussis, an active immunization strategy should be implemented. Some ideas were considered to import Tdap, as well as change the immunization schedule. The immunization schedule of DTaP in Japan is 4 doses in young children only, being one or two times fewer doses in comparison with the schedule of DTaP in the EU and US. The components of Tdap (Adacel and Boostrix) were 2.5–8 μg of PT, 5–8 μg of FHA, 2.5–3 μg of pertactin, 2–2.5 Lf of diphtheria toxoid, and 5 Lf of tetanus toxoid. The five brands of DTaP in Japan have different formulations of components, as shown in Table 1. The B-type DTaP has only two components (Biken and Kaketsu) and T-type vaccines contain several other components besides PT and FHA (Takeda, Denka, and Kitasato). A dose of 0.1 ml of DT was scheduled at the age of 11–12 years. The concentration of tetanus toxoid in 0.2 ml of DTaP was similar to that in 0.1 ml of DT, but that of diphtheria toxoid was higher than that in 0.1 ml of DT. In comparison with Tdap used abroad, 0.2 ml of DTaP contained higher amounts of diphtheria toxoid and there was no significant difference in the incidence of adverse local reactions and serological response. Also, 0.2 ml of DTaP contains lower contents of tetanus toxoid and they induced efficient antibodies against tetanus toxoid. As for the antigen content of pertussis components, the PT antigen content varies from 1.2 to 9.4 μg, and the FHA content from 9.4 to 20.6 μg in 0.2 ml of different brands of DTaP. The GMT of antibodies against PT and FHA showed no significant difference after immunization with 0.2 or 0.5 ml of DTaP, but when comparing the GMT after immunization among different brands with different antigen concentrations, DTaP with higher antigen content did not always induce higher antibody titers. A lower-level serological response was observed in those immunized with a lower antigen content, but sero-positivity (protection levels > 10) was almost 100% after immunization with different bands of DTaP. DTaP with higher antigen content induced more marked serological responses at 4 years of age on booster immunization, but the difference was ten-times for PT antigen and five-times for FHA [33].

In the late 1990s, the resurgence of pertussis might have been associated with multi-factorial events: waning immunity, increased awareness, inappropriate vaccination schedule, improved diagnostic methods, and variant strains evading immunity acquired by immunization [8,34–36]. There have been several reports on mutation of the PT gene and it is still controversial which antigens are related to promoting immunity or reducing the severity of symptom [37,38]. Antibodies against PT reduced susceptibility to pertussis and those against pertactin or Fim2/3 were protective antibodies [39]. Protective immunity was considered to be induced by multiple components [40].

In many developed countries, the control of pertussis is complicated because of the difficulty in case identification, limited persistence of vaccine-acquired immunity, and transmission from unrecognized very mild patients or asymptomatic cases. In Japan, the number of pertussis patients has been increasing and resurgence in very young infant due to household contact was reported [41]. In this report, safe and effective immunization was achieved by 0.2 ml of DTaP instead of 0.1 ml of DT. The booster immunization with pertussis components should be implemented to achieve more effectively control the epidemiology of pertussis in Japan.

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Evaluation of seasonal influenza vaccination effectiveness based on antibody efficacy among the institutionalized elderly in Japan

Megumi Hara[a,*], Keitaro Tanaka[a], Tetsuo Kase[b], Akiko Maeda[c], Yoshio Hirota[c]

[a] Department of Preventive Medicine, Faculty of Medicine, Saga University, 5-1-1 Nabeshima, Saga 849-8501, Japan
[b] Osaka Prefectural Institute of Public Health, 1-3-69 Nakamichi, Higashinari-ku, Osaka 537-0025, Japan
[c] Department of Public Health, Osaka City University Faculty of Medicine, 1-4-3 Asahi-cho, Abeno-ku, Osaka 545-8585, Japan

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ABSTRACT

Influenza vaccination efficacy was evaluated in 114 institutionalized elderly people in 2002/03. Strain A/H3N2 was isolated; 44 and 8 subjects had sudden-onset fever (≥37.8 °C) and kit-diagnosed influenza, respectively. Odds ratios adjusted for age, sex, comorbidity, and vaccine strain (OR_{adj}) were determined using multiple logistic regression. Seroprotected patients (haemagglutination-inhibition antibody titre ≥1:40) had lower incidence of fever (OR_{adj}, 0.35; 95% confidence interval [CI], 0.09–1.28) and kit-diagnosed influenza (OR_{adj}, 0.35; 95% CI, 0.03–4.64) than patients without seroprotection (antibody efficacy, ~65%). Seroprotective levels of vaccination-induced antibodies probably prevent influenza among the institutionalized elderly, although statistical significance could not be confirmed owing to the sample size.

1. Introduction

Influenza vaccination is the primary method worldwide for preventing influenza and its severe complications [1]. Many countries recommend annual influenza vaccination for elderly high-risk individuals in order to induce protection against influenza infection [2]. Because of the yearly occurrence of influenza epidemics, along with the antigenic drift of the virus, it is important to monitor the effectiveness of the vaccine each season. The actual ability of a vaccine to prevent clinical disease must be evaluated on the basis of its efficacy (i.e. the prevention of illness among vaccinated persons in controlled trials) and effectiveness (i.e. the prevention of illness in vaccinated populations) [3]. Studies assessing these factors, however, are often laborious, logistically difficult, or ethically unacceptable. Furthermore, these parameters are difficult to evaluate where vaccination coverage among subjects is high, such as in nursing homes. Consequently, the percentage of subjects achieving a post-vaccination haemagglutination-inhibition (HI) antibody titre ≥1:40 (i.e. the seroprotection rate) is used as a surrogate endpoint that is likely to predict the clinical benefit, i.e. the prevention of influenza and its complications [4]. No prospective design studies that evaluated the effectiveness of influenza vaccines, however, have identified a specific HI antibody titre associated with protection against culture-confirmed influenza. The actual effectiveness of the influenza vaccine is affected by the outbreak size, antigenic similarity between the vaccination strains and the circulating strains, and a variety of host factors. Elderly people with co-existing morbid conditions may be easily susceptible to influenza, even if their antibody levels are identical to those of healthy individuals. Therefore, the extent to which seroprotection afforded by post-vaccination antibodies can prevent influenza among the institutionalized elderly is a matter of great interest. To evaluate this, antibody efficacy—which compares the frequency of illness between those with and those without a protective level of pre-epidemic HI antibodies (≥1:40)—has been proposed [5]; however, this index has rarely been used, due to practical difficulties in confirming the strain-specific disease corresponding to each of the vaccine-induced antibodies. To our knowledge, only one study has used this index in the case of influenza, reporting that antibody efficacy can be considered a sensitive index for evaluating the efficacy of the influenza vaccine [6].

In the present prospective study, we attempted to carefully evaluate antibody efficacy in order to assess the extent to which the effectiveness of the influenza vaccine-induced HI antibody titre ≥1:40 reduced the risk of influenza among the institutionalized elderly during the 2002/03 influenza season.

2. Materials and methods

2.1. Study population

The study was conducted at a nursing home in the Saga Prefecture located in southwestern Japan during the 2002/03 influenza season. The entire study design has been described in detail

* Corresponding author. Tel.: +81 952 34 2289; fax: +81 952 34 2065.
E-mail address: harameg@cc.saga-u.ac.jp (M. Hara).

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previously [7], and a portion of the data from this report was used in this study. This study was approved by the institutional review board associated with Saga University. Of 187 elderly persons in the nursing home, 149 provided written informed consent. None of the subjects had any history of allergy to eggs, past or current neurologic conditions, respiratory illnesses, or fever at the time of vaccination, and all had been vaccinated during the previous influenza season. They were vaccinated subcutaneously with 0.5 ml of commercial trivalent split-virus vaccine (Lot HA025B in 2002, The Research Foundation for Microbial Disease of Osaka University, Osaka) between the 15th and the 31st of October 2002. The 0.5-ml dose of vaccine contained the following antigens: 15 μg each of A/New Caledonia/20/99 (H1N1), A/Panama/2007/99 (H3N2), and B/Shandong/7/97. Both pre- and post-vaccination sera, which were drawn 4–6 weeks after vaccination, were obtained from 114 (30 men and 84 women; mean age range, 66–104 years) of the 149 subjects. Post-vaccination sera could not be obtained from the remaining 35 subjects owing to either subject refusal ($n=28$) or hospital discharge ($n=7$). All serum specimens were stored at −20 °C until assayed.

All participants' body temperatures, respiratory symptoms (cough, sore throat, and nasal congestion), other general symptoms (fever, muscle pain, and general fatigue), hospitalization, discharge, and death were recorded daily from 1 November 2002 to 30 April 2003 in a prospective manner. The details of each febrile episode were ascertained from the admission records of the nursing home in a retrospective fashion. In order to avoid any misclassification of the outcomes, we scrutinized the subjects continuously, and did not include fevers from urinary infection, decubitus ulcers, and/or enteritis in our study outcome.

When the participants suffered any influenza-like symptoms, such as sudden fever (temperature ≥37.8 °C) and respiratory or general symptoms, throat swabs were collected and tested using the Capilia Flu AB rapid diagnosis kit for influenza (Nippon Becton Dickinson Company Ltd., Tokyo, Japan), which utilizes an immunochromatographic method. Eight throat swabs collected from the kit-diagnosed patients were refrigerated in a buffer and sent to our laboratory within 2 d. To confirm the existence and strain of the influenza virus, the circulating virus was cultured by standard methods.

2.2. Laboratory examination

The serum antibody titre of each strain of influenza virus was measured by the HI method [8], using the same antigens as those in the vaccine. Pre- and post-vaccination sera were titrated simultaneously at an outside laboratory (SRL, Tokyo, Japan). The seroconversion rate was defined as the percentage of subjects with either a pre-vaccination HI titre <1:10 and a post-vaccination HI titre ≥1:40 or a pre-vaccination HI titre ≥1:10 and a minimum 4-fold rise in the post-vaccination HI antibody titre [4].

2.3. Influenza surveillance

According to reports from the Infectious Disease Information Center in Saga Prefecture, as recorded by the National Epidemiological Surveillance of Infectious Diseases, an influenza epidemic that was greater than any observed in the previous 10 years was experienced in Saga between 4 November 2002 and 13 April 2003. Two epidemic peaks were observed in the community, and the predominant influenza strains circulating in the study area were A/H3N2 during the first peak (9 December 2002 to 26 January 2003) and B during the second peak (10 February to 30 March 2003). The predominant influenza strain circulating throughout Japan was A/H3N2. Furthermore, only 42% of the influenza A/H3N2 isolates were antigenically similar to the vaccine strain, while in the case of the isolated influenza B virus specimens, the majority were antigenically similar to the vaccine strain [9].

2.4. Statistical analysis

A descriptive statistical analysis, using the Chi-square test and Fisher's exact test, was performed to characterize the study subjects on the basis of the proportion of those with and those without protective levels of post-vaccination HI titres (≥40) according to the vaccine strains. Univariate and multivariate logistic regression modelling were used to obtain crude and adjusted odds ratios (ORs) and 95% confidence intervals (95% CI) of the association of post-vaccination HI titre with febrile illness, febrile illness with symptoms, and kit-diagnosed influenza. These calculations were all conducted using the Statistical Analysis System (SAS) [10]. The antibody efficacy was calculated by the following formula:

$$[1 - (\text{adjusted OR})] \times 100$$

The vaccine's effectiveness is estimated as the product of the antibody's efficacy and the seroconversion rate [6].

3. Results

The distribution of pre- and post-vaccination HI antibody titre among the study subjects has been reported in detail elsewhere [7]. In brief, the proportion of subjects with pre-vaccination HI antibody titre ≥1:40 was 24.6% (17.0–33.5%) for A/H1N1, 56.1% (46.5–65.4%) for A/H3N2, and 10.5% (5.6–17.7%) for B. After vaccination, the proportion of subjects achieving an HI antibody titre ≥1:40 was 61.4% (51.8–70.4%) for A/H1N1, 79.8% (71.3–86.8%) for A/H3N2, and 26.3% (18.5–35.4%) for B, while the proportion of subjects achieving seroconversion for the HI antibody titre was 48.8% (37.9–59.9%), 56% (41.2–70.0%), and 20.6% (13.2–29.7%), respectively.

The characteristics of our subjects are shown according to the proportion of those with and those without a protective level of post-vaccination HI titre (≥40) and the vaccine strains in Table 1. The proportion of pulmonary disease was higher among those without a protective level of HI titre for A/H3N2 than among those with a protective level. The distributions of age, sex, and other chronic conditions did not differ with the post-vaccination HI titre against any vaccine strain.

During the follow-up period, 12 hospitalizations (4 cases of pneumonia, 3 fractures, 2 cases of aggravation of chronic conditions, 1 stroke, and 2 due to other causes), 6 deaths (3 due to pneumonia, 2 due to old age, and 1 due to other causes), and 3 discharges occurred. Forty-four subjects experienced sudden-onset fever (temperature ≥37.8 °C), and 27 of these had symptoms (1 or more) such as cough, sore throat, nasal congestion, and general fatigue with fever. Among the 27 symptomatically diagnosed patients, 8 tested positive for type A influenza by using a rapid diagnosis kit. To confirm the virus strain, we examined 8 swabs collected from kit-diagnosed patients; 2 of these were virologically confirmed as A/H3N2 influenza. The other circulating strains were not detected in the study subjects.

The ORs for febrile illness and kit-diagnosed influenza according to the baseline characteristics of the study participants are shown in Table 2. Old age increased the risk of fever and fever with symptoms, and the presence of hypertension led to a reduced risk. Similar associations were observed for kit-diagnosed influenza, although no statistical significance was detected. The other factors were not associated with the risk of any outcomes.

The ORs for febrile illness and kit-diagnosed influenza according to the vaccine strains are shown in Table 3. In comparison to subjects without a protective level of HI titre (<40), those with a seroprotective level of post-vaccination HI antibody titre (≥40) to

Table 1
Characteristics of the study subjects during the pre-epidemic 2002/03 influenza season.

Post-vaccination	A/H1N1		A/H3N2		B	
HI titre	≥40	<40	≥40	<40	≥40	<40
Number (%)	70(61.4)	44(38.6)	91(79.8)	23(20.2)	30(26.3)	84(73.7)
Age in years (%)						
65–79	21(30.0)	10(22.7)	26(28.6)	5(21.7)	8(26.7)	23(27.4)
80–89	37(52.9)	26(59.1)	49(53.9)	14(60.9)	17(56.7)	46(54.8)
90+	12(17.1)	8(18.2)	16(17.6)	4(17.4)	5(16.7)	15(17.9)
Female (%)	53(75.7)	31(70.5)	68(74.7)	16(69.6)	24(80.0)	60(71.4)
Chronic conditions (%)						
Heart disease	34(49.3)	22(50.0)	46(51.1)	10(43.5)	17(56.7)	39(50.0)
Pulmonary disease	9(13.0)	8(18.6)	9(10.1)	8 (34.8)[a]	5(16.8)	12(14.6)
Diabetes	5(7.3)	1(2.3)	6(6.7)	0(0.0)	1(3.3)	5(6.0)
Hypertension	28(40.6)	16(36.4)	34(37.8)	10(43.5)	12(40.0)	32(38.6)
Stroke	19(27.6)	18(40.9)	29(32.2)	8(34.8)	9(30.0)	28(33.7)
Other	24(35.3)	14(31.8)	32(36.0)	6(26.1)	13(43.3)	25(30.5)
Serum albumin <3.5 g/dl	11(15.7)	8(18.2)	16(17.6)	3(13.0)	6(20.0)	13(15.5)

[a] Significantly different between subjects with and without a protective level HI titre ($P < 0.01$ by the Chi-square test).

A/H3N2—the strain detected in our study—showed decreased risks of febrile illness and kit-diagnosed influenza, although no statistical significance was noted. On the other hand, a protective level of post-vaccination HI titres to A/H1N1 and B did not show such associations. In the multivariate analysis, the vaccine strain and possible confounders were included; model 3 included age, sex, and hypertension, which was significantly associated with fever; in addition, model 4 included pulmonary disease, which was significantly associated with the seroprotection rate of A/H3N2. A protective level of HI antibody titres to A/H3N2 decreased the risks of fever (OR_{adj}, 0.35; 95% CI: 0.09–1.28) and kit-diagnosed influenza (OR_{adj}, 0.35; 95% CI: 0.03–4.64). When the outcome was limited to fever with symptoms, a decreased OR_{adj} was observed for A/H3N2, although the degree of decrease was lesser than that in the case of the other outcomes.

The antibody efficacy ($1 - OR_{adj}$) against fever related to A/H3N2 was thus estimated to be 65% on the basis of fully adjusted ORs for fever. Similarly, the antibody efficacy for kit-diagnosed influenza

Table 2
Odds ratios (ORs) and 95% confidence intervals (CI) for fever (≥37.8 °C), fever with symptoms (cough, nasal congestion, and sore throat), and kit-diagnosed influenza during the survey period according to the baseline characteristics of the study subjects.

	Total number	Fever ≥37.8 °C			Fever ≥37.8 °C with symptoms			Kit-diagnosed influenza		
		n	OR	95% CI	*n*	OR	95% CI	*n*	OR	95% CI
Age in years										
65–79	31	8	1		4	1		0		
80–89	63	25	1.89	0.73–4.89	15	2.11	0.64–7.00	5	1	
90+	20	11	3.51	1.07–11.59	8	4.50	1.13–17.88	3	3.14	0.67–14.40
Gender		*P* for trend: *P* = 0.12			*P* for trend: *P* = 0.10			*P* for trend: *P* = 0.14		
Male	30	16	1		9	1		1	1	
Female	84	28	0.44	0.19–1.02	18	0.64	0.25–1.63	7	2.64	0.31–22.36
Chronic conditions										
Heart disease										
No	58	17	1		10	1		2	1	
Yes	56	27	1.57	0.68–3.64	17	1.45	0.57–3.68	6	2.37	0.45–12.47
Pulmonary disease										
No	97	38	1		22	1		7	1	
Yes	17	6	0.53	0.17–1.69	5	1.01	0.30–3.32	1	0.58	0.07–5.09
Diabetes										
No	108	42	1		26	1		8	1	
Yes	6	2	0.59	0.09–3.82	1	0.50	0.05–4.76	0	Not calculated	
Hypertension										
No	70	34	1		23	1		7	1	
Yes	44	10	0.40	0.16–0.99	4	0.26	0.08–0.85	1	0.29	0.03–2.49
Stroke										
No	77	31	1		17	1		5	1	
Yes	37	13	0.80	0.33–1.94	10	1.40	0.53–3.65	3	1.34	0.30–6.08
Other diseases										
No	76	30	1		18	1		4	1	
Yes	38	14	0.77	0.32–1.84	9	0.88	0.34–2.31	4	2.08	0.48–9.13
Serum albumin										
≥3.5 g/dl	95	36	1		20	1		5	1	
<3.5 g/dl	19	8	1.48	0.48–4.56	7	3.02	0.89–10.27	3	4.80	0.92–25.23

Table 3
Odds ratios (ORs) and 95% confidence intervals (95% CI) for febrile illness and kit-diagnosed influenza, when comparing lower and higher pre-epidemic HI antibody titres according to vaccine strains.

	HI titer	Total number	*n*	Model 1		Model 2		Model 3		Model 4	
				OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Fever (≥37.8 °C) with sudden onset											
A/H1N1	<40	44	16	1		1		1		1	
	≥40	70	28	1.22	0.52–2.85	1.40	0.55–3.58	1.77	0.64–4.90	1.83	0.65–5.17
A/H3N2	<40	23	10	1		1		1		1	
	≥40	91	34	0.65	0.23–1.82	0.55	0.18–1.69	0.48	0.14–1.59	0.35	0.09–1.28
B	<40	84	29	1		1		1		1	
	≥40	30	15	1.39	0.56–3.46	1.33	0.52–3.40	1.38	0.51–3.77	1.44	0.52–3.98
Fever ≥ 37.8 °C with symptoms (cough, nasal congestion, and sore throat)											
A/H1N1	<40	44	9	1		1		1		1	
	≥40	70	18	1.42	0.55–3.68	1.38	0.48–3.96	1.69	0.55–5.21	1.70	0.55–5.27
A/H3N2	<40	23	5	1		1		1		1	
	≥40	91	22	1.05	0.33–3.36	0.90	0.25–3.21	0.63	0.15–2.61	0.61	0.14–2.60
B	<40	84	17	1		1		1		1	
	≥40	30	10	1.47	0.55–3.88	1.38	0.51–3.73	1.39	0.49–3.99	1.39	0.49–4.00
Positive for type A influenza by using rapid diagnosis kit											
A/H1N1	<40	44	1	1		1		1		1	
	≥40	70	7	4.86	0.57–41.28	7.19	0.64–81.15	5.46	0.49–61.35	5.29	0.49–56.74
A/H3N2	<40	23	2	1		1		1		1	
	≥40	91	6	0.67	0.12–3.66	0.29	0.04–2.19	0.36	0.03–4.71	0.35	0.03–4.64
B	<40	84	4	1		1		1		1	
	≥40	30	4	2.30	0.53–10.03	1.67	0.36–7.86	1.65	0.31–8.78	1.70	0.32–9.16

n: number of outcomes. Model 1: crude, model 2: adjusted for vaccine strains, model 3: adjusted for all variables in model 2, plus age, sex, and hypertension, model 4: adjusted for all variables in model 3, plus pulmonary disease.

was estimated to be 65%. In the present study, 50 subjects did not have a protective level of HI titre (≥40) to A/H3N2, as noted from their pre-vaccination sera, and 28 of these subjects achieved protective levels post-vaccination. The seroconversion rate was thus 56% (28/50). The vaccine efficacy [6], which is the product of the antibody efficacy and the seroconversion rate, was expected to be 36%.

4. Discussion

Although little is known regarding the associations between vaccine strain-specific HI antibody titre and protection against influenza, the percentage of subjects achieving an HI antibody titre (≥1:40) after vaccination has been used as a surrogate endpoint that is likely to predict clinical benefits. Few studies have suggested that protection from influenza increases with a higher HI antibody titre [6,11], and Hirota et al. reported that the protective levels of antibodies against the circulating virus strain showed a significantly decreased risk of ILI among healthy adults (OR, 0.14), further suggesting that antibody efficacy (estimated to be 86%) is a sensitive index for evaluating the effectiveness of the influenza vaccine [6]. In this study, we first reported the influenza vaccine's antibody efficacy among the elderly. We observed that the protective level of pre-epidemic HI antibody (≥1:40) after vaccination for the circulating virus strain (A/H3N2) was associated with a reduction in the incidence of influenza-related fever (OR_{adj}, 0.35; 95% CI: 0.09–1.28) and kit-diagnosed influenza (OR_{adj}, 0.35; 95% CI: 0.03–4.64) during an epidemic period among the institutionalized elderly, and the antibody efficacy against fever related to A/H3N2 influenza was estimated to be 65%, although no statistical significance was detected, due to the limited sample size.

The efficacy and effectiveness of the influenza vaccine depend in part on the age and immune-competence of the vaccine's recipient, the degree of similarity between the viruses in the vaccine and those in circulation, and the outcomes being measured [3]. In general, the vaccine's effectiveness in preventing influenza is reported to be lower among the elderly [12–14], due to a diminished immune response after vaccination [3,7,15–17]. According to the Advisory Committee on Immunization Practice (ACIP) in the US, an inactivated influenza vaccine prevents influenza in approximately 70–90% of healthy adults aged <65 years, while such prevention is only seen in 30–40% of the elderly in nursing homes, even when the vaccine and the circulating viruses are antigenically similar [18]. The value of the vaccine efficacy in the present study was estimated to be 36%, which appears to be comparable to that of previous reports. The antibody efficacy method is considered to be useful in such assessments because it can be calculated from the information concerning the vaccinees alone.

Along with the vaccine's efficacy and effectiveness, the antibody efficacy is also influenced by the degree of similarity between the vaccine strains and the epidemic virus, as well as the outcomes being measured. Therefore, it would be difficult to directly compare the antibody efficacy observed in our study and those of other studies. However, our finding that the antibody efficacy among the elderly (65%) was much lower than that among healthy adults (86%) [6] suggests that a suitable cut-off level for a post-vaccination seroprotective HI antibody titre among the elderly remains to be elucidated. de Jong et al. reassessed all the influenza vaccination data published for healthy children or young adults from 1966 to 1985 and suggested that HI antibody titres ranging from 1:15 to 1:65 may be associated with protection from illness in 50% of the subjects. Furthermore, they pointed out that none of the studies were performed with elderly or other high-risk subjects who are the main target population for the influenza vaccination [11]. Further studies among the elderly concerning this issue are urgently needed.

Several limitations of this study, including misclassification and the acknowledgement of power issues, should be mentioned. First,

misclassification of diagnoses, especially differential misclassification, affects the evaluation of influenza vaccine effectiveness. To avoid differential misclassification, study participants must be equally scrutinized using identical criteria; such equal observation is more important than the specificity of the diagnosis [19]. The sudden-onset fever and kit-diagnosed influenza in this study were detected by active surveillance, but their details were retrospectively checked using clinical records. Therefore, some bias might have occurred in the information about the symptoms owing to differences in an individual's subjective complaints, the frequency of contact with the workers at the nursing homes, and the type of workers that they came in contact with (doctors, nurses, health care workers, and others). In the present study, fever alone or kit-diagnosed influenza might be better outcomes for evaluating effectiveness than fever with symptoms, because differential misclassifications cause more complicated and serious consequences. To avoid non-differential diagnosis misclassification, the study period should be limited to the peak period [19]. Regarding the peak of an influenza epidemic, there was no obvious peak for any of the outcomes in the nursing home, despite the fact that 2 epidemic peaks were observed in the community. Limiting the study period to the peak period appeared to be inappropriate for the present study, because the elderly individuals included here were mostly isolated from the community. Second, because of the limited sample size, the statistical power was not high enough to detect the significance of the vaccine's effectiveness against influenza-related fever and kit-diagnosed influenza. In addition, the vaccine's effectiveness against pneumonia, hospitalization, and death could not be evaluated. However, we scrutinized each participant equally throughout the influenza season, and such close follow-up reduced the misclassification of outcomes described above and enabled us to calculate the ORs even in a relatively small sample size. In addition, the active outcome findings were successfully observed by a prospective follow-up, and the circulating virus strain in the nursing homes was virologically confirmed, enabling us to estimate the strain-specific antibody efficacy. Only post-vaccination antibodies to the A/H3N2 strain, i.e. the detected strain, were protective against fever and kit-diagnosed influenza, which indicates that they were caused by circulating strains that were antigenically similar to the strain included in the vaccine. It may be argued that we should use culture-confirmed influenza as an outcome; however, it is considered that the performance of virus culture examinations only becomes meaningful after thorough case detection has been conducted [20]. For instance, Belshe et al. achieved almost perfect case detection in a randomised control trial, which is one of the most comprehensive studies on influenza vaccine efficacy reported to date, with 109 culture-positive cases being identified from 3005 specimens collected from symptomatic subjects [21].

In conclusion, the results of our study lend support to the usefulness of antibody efficacy, and show the extent to which the effectiveness of an influenza vaccine-induced HI antibody titre $\geq$1:40 reduces the risk of influenza among the elderly residing in nursing homes.

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Inactivated influenza vaccine effectiveness against influenza-like illness among young children in Japan—With special reference to minimizing outcome misclassification

Hirotaka Ochiai[a,*,1], Megumi Fujieda[a,1], Satoko Ohfuji[a], Wakaba Fukushima[a], Kyoko Kondo[a], Akiko Maeda[a], Takashi Nakano[b], Hitoshi Kamiya[b], Yoshio Hirota[a], for the Influenza Vaccine Epidemiology Study Group[2]

[a] Department of Public Health, Osaka City University Graduate School of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka 545-8585, Japan
[b] National Mie Hospital, 357, Ozato-Kubota, Tsu, Mie 514-0125, Japan

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ABSTRACT

The aim of the present study was to investigate the influenza vaccine effectiveness among young children in Japan. Study subjects were recruited from 43 pediatric clinics. Influenza-like illness (ILI) was defined as an acute febrile illness with respiratory symptoms; ILI with a fever of ≥39 °C was considered to be severe ILI (SILI). The adjusted OR of vaccination significantly decreased to 0.75 for SILI. Influenza vaccination for young children had a protective effect on the occurrences of SILI. This study also indicated that three key tools (case surveillance with equal scrutiny, confining observation to the peak epidemic period, and adoption of strict criteria for ILI) could minimize outcome misclassification and thus provide adequate methodology for monitoring vaccine effectiveness without laboratory confirmation.

1. Introduction

Although influenza viruses can cause disease among persons in any age group [1–3], rates of influenza virus infection are highest among children [4]. It has been reported that children aged 6–23 months are at substantially increased risk for influenza-related hospitalizations, and those aged 24–59 months are at increased risk for influenza-related clinic and emergency department visits [5].

A number of studies have investigated the effectiveness or efficacy of the influenza vaccine among young children [6–14]. However, these results are not consistent. Some studies failed to detect vaccine effectiveness even though applying case surveillance with laboratory confirmation, while other studies reported vaccine effectiveness of 24–69%.

Furthermore, studies conducted in Japan to date have been too limited. In Japan, the vaccine dose for young children (<1 year old, 0.1 ml; 1–5 years, 0.2 ml; 6–12 years, 0.3 ml; 13 years or over, 0.5 ml of trivalent vaccine including 30 μg haemagglutinin/ml of each strain) is smaller than that in the USA or Europe and it is disputable whether this dose leads to sufficient seroresponse. Therefore, it is important to evaluate the vaccine effectiveness of the current dose among young children in Japan.

Accordingly, we precisely analyzed data collected among Japanese children under 6 years of age during the 2000–2001 season to assess influenza vaccine effectiveness. Then, the effect of outcome misclassification was thoroughly considered by using several outcomes and confining observation to the peak epidemic period.

2. Materials and methods

2.1. Study subjects

Subjects comprised children under 6 years of age recruited from 43 pediatric clinics, where this study group members belonged, in seven different areas of Japan: (from north to south) Hokkaido (2 clinics), Iwate (12 clinics), Tokyo (6 clinics), Mie (7 clinics), Osaka (2 clinics), Shikoku (10 clinics), and Fukuoka (4 clinics).

Children who received an influenza vaccine at each clinic upon parental request (vaccinee) were entered into the vaccinated group. Then, one or two children who visited the pediatrician subsequent to each vaccinee and whose parents did not request to have their children vaccinated (nonvaccinee) were enrolled into the unvaccinated group. A total of 2353 children

* Corresponding author at: Department of Public Health, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan. Tel.: +81 3 3784 8134; fax: +81 3 3784 7733.
E-mail address: h-ochiai@med.showa-u.ac.jp (H. Ochiai).
[1] Both authors contributed equally to this work.
[2] Other members of the Study Group are listed in Appendix A.

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(1216 vaccinees and 1137 nonvaccinees) were enrolled in this study.

Vaccinations were performed using commercial inactivated influenza vaccine containing A/New Caledonia/20/99 (H1N1), A/Panama/2007/99 (H3N2), and B/Yamanashi/166/98. These vaccines included 30 μg of haemagglutinin per 1.0 ml from each strain. Each dose was 0.1 ml for children under 1 year of age or 0.2 ml for children aged 1.0–5.9 years, in accordance with the standard recommendations for vaccine use in Japan. Two doses of vaccine were commonly administered subcutaneously 2–4 weeks apart.

Informed consent to participate in the study was obtained from all parents or guardians. This study was approved by the institutional review board associated with the Osaka City University Graduate School of Medicine.

2.2. Information collection

At the time of enrollment, the following information was obtained by means of a self-administered questionnaire completed by each child's parent or guardian: sex, date of birth, preschool attendance, number of family members, number of siblings and number of rooms in the home. Information on the following health-related conditions of each child was collected from his or her pediatrician by using a structured questionnaire: vaccination status, vaccination date if vaccinated, current body weight, underlying illnesses (i.e., heart disease, renal disease, anemia, tonsillitis, atopy, allergic symptoms), influenza vaccination during the previous season, and disease onset during the previous influenza season.

As a follow-up survey, each subject's parent or guardian was asked to complete weekly postal questionnaires about the child's maximum body temperature (°C) and three symptoms (runny nose or nasal congestion, sore throat, and cough) during the preceding week. This questionnaire was to be returned to the pediatrician's office each week during the follow-up period (15 weeks) from the 51st week of 2000 (December 17–23) to the 13th week of 2001 (March 25–31). Concurrently, each pediatrician was also asked to make keep weekly records of the subject's visit to the medical office, including the date of any visit and the presence or absence of any of four complications (pneumonia, encephalopathy, encephalitis, and convulsion).

2.3. Outcome definition

The following three outcomes were used for assessing the influenza vaccine effectiveness: influenza-like illness (ILI); medical office visits (MOV) for any cause; and medical office visits for severe influenza-like illness (SILI-related MOV). ILI was defined as an acute febrile illness with one or more symptoms (runny nose or nasal congestion, sore throat, cough), while ILI with a fever of ≥39 °C was considered to be severe ILI (SILI). All MOV were identified from the pediatrician's report. The case who had simultaneously reported both SILI and MOV was regarded as SILI-related MOV.

2.4. The peak epidemic period of influenza

The peak epidemic period of influenza was determined using the following two surveillance data in each area [6,15]: the weekly number of clinical influenza cases reported by the sentinels and the weekly number of influenza virus isolates at Prefectural Public Health Laboratories in the National Influenza Surveillance System. Eventually, the period in Hokkaido, Tokyo, Mie, Osaka, and Shikoku was determined to be from the 7th to 13th weeks of 2001 (February 11 to March 31) and that in Iwate and Fukuoka was determined to be from the 9th to 13th weeks of 2001 (February 25 to March 31).

2.5. Data analyses

The Chi-squared or Fisher's exact test and the Wilcoxon rank-sum test were used to compare various characteristics between vaccinees and nonvaccinees. To calculate the odds ratio (OR) and 95% confidence interval (95% CI) of vaccination for each outcome, a logistic regression model was employed with potential confounders. Variables that were different between vaccinees and nonvaccinees with P values less than 0.1 or that seemed to be medically related to disease were put in the model. For adjustment, age was included in the model as a categorical variable (six classes with 1-year age intervals) rather than as a continuous variable, by which the effect of vaccine dose can be simultaneously controlled. Vaccine effectiveness (VE) is equivalent to (1 − adjusted OR) × 100%. These calculations were all conducted using Statistical Analysis System (SAS, Version 9.1).

3. Results

Among 2353 subjects, 69 subjects were excluded because of incomplete data in the follow-up survey. (There was no substantial difference in the frequency of the excluded subjects between the groups: 3% (31/1216) in vaccines and 3% (38/1137) in nonvaccinees; $P = 0.255$.) Another 19 subjects were subsequently found to have received only a single dose of vaccine. Thus, data from a total of 2265 subjects were analyzed.

The mean (median) ± standard deviation age of the 2265 subjects was 3.0 (2.9) ± 1.5 years. Most children (over 60%) were between the ages of 1 and 3 years. The proportion of children under 1 year of age was less than 10%.

Table 1
Baseline characteristics of study participants according to vaccination status.

Characteristics	Vaccinee (n = 1166)	Nonvaccinee (n = 1099)	P value[a]
Male (%)	51.2	51.0	0.907
Age (years)	3.1 (3.2)	2.6 (2.7)	<0.001
Current body weight (kg)	14.0 (14.0)	12.8 (13.1)	<0.001
Preschool attendance (%)	50.7	44.7	0.004
Number of family members	4.0 (4.3)	4.0 (4.2)	0.740
Siblings	2.0 (2.03)	2.0 (1.99)	0.306
Influenza vaccination during the previous season (%)	38.1	2.2	<0.001
Disease onset during the previous influenza season (%)	13.1	10.7	0.066
Underlying illnesses (%)			
Tonsillitis	2.1	5.3	<0.001
Atopy	7.1	11.7	<0.001
Allergy	5.9	10.0	<0.001

Except where indicated percentage (%), values are median (mean).
[a] Chi-squared test or Wilcoxon rank-sum test.

Table 2
Odds ratios of vaccination for ILI with different fever levels, MOV for any cause, and SILI-related MOV during the entire follow-up period.

Outcome	Number (%)		Crude		Adjusted[a]	
	Vaccinee (n = 1166)	Nonvaccinee (n = 1099)	OR (95% CI)	P value	OR (95% CI)	P value
ILI with different fever levels						
≥37.0 °C	862 (74)	842 (77)	0.87 (0.72–1.05)	0.139	0.95 (0.75–1.19)	0.640
≥37.5 °C	792 (68)	756 (69)	0.96 (0.81–1.15)	0.658	1.08 (0.88–1.34)	0.463
≥38.0 °C	676 (58)	663 (60)	0.91 (0.77–1.07)	0.255	1.03 (0.84–1.26)	0.770
≥39.0 °C (SILI)	338 (29)	373 (34)	0.80 (0.67–0.95)	0.011	0.87 (0.71–1.08)	0.204
MOV for any cause	578 (50)	596 (54)	0.83 (0.70–0.98)	0.027	0.79 (0.64–0.98)	0.028
SILI-related MOV	171 (15)	205 (19)	0.75 (0.60–0.94)	0.011	0.73 (0.56–0.96)	0.023

OR, Odds ratio; CI, confidence interval; ILI, influenza-like illness; MOV, medical office visits; SILI, severe ILI.
[a] Adjusted for age (categorical variable), sex, current body weight, preschool attendance, siblings, tonsilitis, atopy, allergy, influenza vaccination during the previous season, disease onset during the previous influenza season, area.

The baseline characteristics of vaccinees and nonvaccinees are shown in Table 1. Factors that were thought to be associated with a decrease in disease risk, such as older age, heavier body weight, and influenza vaccination during the previous season, were more frequently found in the vaccinated group. Children attending preschool, who are more exposed to viruses and thereby have a high risk of disease, were also more frequently found in the vaccinated group. However, children who had tonsillitis, atopy, or allergy were more frequently found in the nonvaccinated group.

Table 2 shows the crude and adjusted ORs for ILI with different fever levels, MOV for any cause, and SILI-related MOV during the entire follow-up period. Adjusted ORs for ILI at each fever level were not statistically significant, whereas influenza vaccination revealed significantly decreased ORs for MOV for any cause (adjusted OR, 0.79; 95% CI, 0.64–0.98) and for SILI-related MOV (adjusted OR, 0.73; 95% CI, 0.56–0.96).

Next, the crude and adjusted ORs were calculated for each outcome event confined into the peak epidemic period (Table 3). A higher level of fever brought about a lower OR of vaccination for ILI. The adjusted OR for ILI with a fever ≥39 °C reached a statistically significant level (OR, 0.75; 95% CI, 0.58–0.97), from which the VE was estimated to be 25% (3–42%). Vaccination also illustrated the decreased ORs for MOV for any cause (adjusted OR, 0.80; 95% CI, 0.64–0.98) and for SILI-related MOV (adjusted OR, 0.68; 95% CI, 0.48–0.96). However, the point estimate and 95% CI for MOV for any cause were nearly the same as those observed during the entire follow-up period. In contrast, the decreases in ORs for SILI and SILI-related MOV were more pronounced, as compared to the observation during the entire follow-up period.

The VE for SILI and SILI-related MOV were evaluated for the different age categories (Table 4). Among children under 1 year of age, decreased ORs were not observed for SILI and SILI-related MOV (data not shown). When analysis was performed among children aged 1.0–5.9 years by 1-year intervals, all ORs were less than unity, regardless of statistical significance level, except for that of SILI among children aged 4.0–4.9 years. Significant ORs for SILI and/or SILI-related MOV were observed: for children aged 3.0–3.9 years, the OR for SILI was 0.50 (0.26–0.96) and the OR for SILI-related MOV was 0.38 (0.15–0.97); for children aged 1.0–1.9 years, the OR for SILI was 0.56 (0.33–0.95).

4. Discussion

4.1. VE among children under 6 years of age

This prospective study showed that influenza vaccination for young children had a protective effect on the occurrence of SILI and SILI-related MOV. The VE were 25% (95% CI, 3–42%) for SILI and 32% (95% CI, 4–52%) for SILI-related MOV. These VEs were lower than those in previous studies [10,12,13]. The following interpretations could explain this discrepancy. First, the determination of ILI as the outcome in this study was less specific for influenza diagnosis compared with laboratory-confirmed diagnoses. Therefore, the ILI group inevitably included noninfluenza illnesses, which may have caused outcome misclassification. However, this misclassification can be considered nondifferential between vaccinees and nonvaccinees and would result in an underestimation of VE. Another possible interpretation is the antigenic match between vaccine strain and circulating virus strain. During the 2000–2001 influenza season, influenza virus isolates reported from Prefectural Public Health Laboratories across the country included 2280 cases of type B (46%), 1862 cases of type H1N1 (38%), and 803 cases of H3N2 (16%) [16]. However, only 13% of the most circulating influenza B viruses was antigenically similar to the vaccine strain, although 80% of the H1N1 virus and 92% of the H3N2 virus were well matched to the vaccine strain. This might account for the lower VE estimates in this study. Furthermore, the lower VE in this study might be explained to some extent by the vaccine dose in Japan, which is smaller than that in other countries.

Table 3
Odds ratios of vaccination for ILI with different fever levels, MOV for any cause, and SILI-related MOV during the peak epidemic period of influenza.

Outcome	Number (%)		Crude		Adjusted[a]	
	Vaccinee (n = 1166)	Nonvaccinee (n = 1099)	OR (95% CI)	P value	OR (95% CI)	P value
ILI with different fever levels						
≥37.0 °C	562 (48)	579 (53)	0.84 (0.71–0.99)	0.033	0.91 (0.75–1.13)	0.370
≥37.5 °C	472 (40)	504 (46)	0.80 (0.68–0.95)	0.010	0.89 (0.73–1.09)	0.262
≥38.0 °C	398 (34)	425 (39)	0.82 (0.69–0.98)	0.025	0.88 (0.72–1.08)	0.217
≥39.0 °C (SILI)	184 (16)	231 (21)	0.70 (0.57–0.87)	0.001	0.75 (0.58–0.97)	0.031
MOV for any cause	401 (34)	429 (39)	0.82 (0.69–0.97)	0.022	0.80 (0.64–0.98)	0.035
SILI-related MOV	89 (8)	119 (11)	0.68 (0.51–0.91)	0.009	0.68 (0.48–0.96)	0.027

OR, Odds ratio; CI, confidence interval; ILI, influenza-like illness; MOV, medical office visits; SILI, severe ILI
[a] Adjusted for age (categorical variable), sex, current body weight, preschool attendance, siblings, tonsilitis, atopy, allergy, influenza vaccination during the previous season, disease onset during the previous influenza season, area.

Table 4
Adjusted odds ratios of vaccination for SILI and SILI-related MOV during the peak epidemic period of influenza by age category.

Age category (years)	SILI		SILI-related MOV	
	Cases (%)	AOR (95% CI)	Cases (%)	AOR (95% CI)
1.0–1.9[a]				
Vaccinee (n=243)	34 (14)	0.56 (0.33–0.95)	18 (7)	0.79 (0.40–1.56)
Nonvaccinee (n=271)	65 (24)	1.00	32 (12)	1.00
2.0–2.9[a]				
Vaccinee (n=242)	34 (14)	0.56 (0.29–1.07)	18 (7)	0.60 (0.25–1.41)
Nonvaccinee (n=191)	40 (21)	1.00	23 (12)	1.00
3.0–3.9[a]				
Vaccinee (n=229)	26 (11)	0.50 (0.26–0.96)	13 (6)	0.38 (0.15–0.97)
Nonvaccinee (n=188)	45 (24)	1.00	24 (13)	1.00
4.0–4.9[a]				
Vaccinee (n=226)	50 (22)	1.32 (0.71–2.47)	25 (11)	0.92 (0.40–2.11)
Nonvaccinee (n=173)	28 (16)	1.00	16 (9)	1.00
5.0–5.9[a]				
Vaccinee (n=169)	26 (15)	0.74 (0.34–1.60)	10 (6)	0.34 (0.11–1.05)
Nonvaccinee (n=115)	22 (19)	1.00	13 (11)	1.00

AOR, Adjusted odds ratio; CI, confidence interval; ILI, influenza-like illness; MOV, medical office visits; SILI, severe ILI

[a] Adjusted for sex, current body weight, preschool attendance, siblings, tonsilitis, atopy, allergy, influenza vaccination during the previous season, disease onset during the previous influenza season, area.

4.2. VE according to age category

This study did not detect VE among children under 1 year of age. A recent study reported that the immune response to influenza vaccine is lower among children under 1 year of age than among children aged 1–3 years [17]. It has also been shown that influenza epidemics often overlap with the circulation of respiratory syncytial virus [18], which has a greater health impact in very young children than in older children [19]. Therefore, ILI defined to measure VE could be more influenced by noninfluenza illnesses among younger children than among older children, causing the failure to detect VE in children under 1 year of age. Furthermore, the sample size of very young children in this study might be too small to clearly demonstrate VE.

When age groups were combined to include children between the ages of 1 and 5 years and children between the ages of 2 and 5 years, statistical significance was observed among these groups (data not shown). In the analysis by 1-year intervals, the ORs were less than unity in almost all age categories, although no significant decrease in OR was detected in most categories. The main reason for this is loss of statistical power as shown by the wide confidence interval, as these results were based on only a small segment of the total subject population.

4.3. Control of outcome misclassification

When ILI, which is less specific to influenza compared to laboratory confirmation, is used as the study outcome, misclassification due to noninfluenza illnesses must be carefully considered. In the present study, the following three methods were used to minimize the effect of outcome misclassification.

First, we prospectively collected information on maximum body temperature and symptoms each week during the entire follow-up period using a postal questionnaire, which enabled us to follow all study subjects with equal intensity. This method leads to nondifferential rather than differential outcome misclassification, if any, and therefore underestimates VE. Even if laboratory-confirmed influenza diagnoses, which are more specific than ILI, had been used as a study outcome, passive case findings only from clinic visitors with influenza would result in a complicated bias. For example, one study has reported that in a cohort study, vaccinees may more readily visit a clinic and may be more likely to report ILI symptoms to a physician than nonvaccinees because they may be more health conscious. This would lead to an increased number of patients with influenza in the vaccinee group, which would affect the validity of the study result. Therefore, case surveillance with equal scrutiny of both vaccinees and nonvaccinees is more essential than the specificity of diagnosis [20].

Second, observation of ILI was limited to the peak epidemic period by using local surveillance data. During the peak period, consistently decreased ORs for ILI with different levels of fever were observed in comparison with those during the entire follow-up period. Similarly, a more pronounced OR for SILI-related MOV was obtained after confining observation to the peak epidemic period (OR, 0.73–0.68). These results suggested that confining observation to the peak epidemic period could be the key method to minimize the inclusion of noninfluenza cases in ILI diagnosis.

Third, to evaluate VE for ILI, several cut-off points for fever in cases of ILI were used. As shown in Table 3, higher level of fever brought about the lower OR of vaccination for ILI (OR, 0.91–0.75). These results could be interpreted to mean that an increasing fever level makes ILI more specific to influenza, suggesting that adoption of strict criteria for ILI could minimize outcome misclassification when laboratory confirmation is not performed.

4.4. Limitation

This study had a nonrandomized design in which vaccination or nonvaccination was self-selected by the parents. As some baseline characteristics were significantly different between vaccinees and nonvaccinees and the ORs of vaccination varied by each area, the effect of potential confounders was taken into consideration in multivariate analysis. However, the possibility of residual confounding is not deniable. In addition, the study subject comprised children recruited from 43 clinics that this study group members belonged, which might limit generalizability to other population.

It is possible that some children might not have visited their pediatrician even though they had contracted influenza. If the behavior of seeking medical care was different between vaccinees and nonvaccinees, serious bias would have been introduced. To examine this propensity, the proportion of MOV was calculated separately for vaccinees and nonvaccinees among all SILI cases during the peak epidemic period. The results demonstrated no substantial difference in the frequency of MOV between the groups:

48% (89/184) in vaccinees and 52% (119/231) in nonvaccinees ($P = 0.524$). However, such propensity should be always considered for careful interpretation when using MOV as an outcome.

In this study, the antibody titer was not measured. However, previous study reported the influenza vaccine efficacy against serologically confirmed influenza (rise in antibody titres in the post-vaccine phase) among children [21]. Furthermore, the study in Japan showed that the seroprotection proportion (postvaccination titer $\geq$ 1:40) after 2 doses of vaccine in children aged 1.0–3.9 years was over 50% against A(H1) or A(H3) [17]. Therefore, this study results could be reasonable.

5. Conclusion

In summary, influenza vaccination for young children had a protective effect on the occurrences of SILI and SILI-related MOV. However, VE was not clearly shown in very young children (under 1 year of age). This study indicated that three key tools (case surveillance with equal scrutiny, confining observation to the peak epidemic period, and adoption of strict criteria for ILI) could minimize outcome misclassification and thus provide adequate methodology for monitoring VE in different seasons and/or populations, without laboratory confirmation.

Appendix A.

Space limitations preclude the inclusion as authors of the following members of the Influenza Vaccine Epidemiology Study Group:

Drs. Tatsuru Yamanaka and Haruhiko Wataya, Hokkaido Prefecture; Drs. Yuhei Takasago, Mitsuo Kamihara, Yukiko Usui, Shuka Watanabe, Toshiko Yamaguchi, Shinji Yoshida, Asaka Nishijima, Tsuneji Kanno, Hisao Nagaoka, Hiroyasu Wada, Eiji Ogawa and Keiko Onodera, Iwate Prefecture; Drs. Kazuo Ito, Akira Aizawa, Takamitsu Matsudaira, Kinjiro Kodaira, Takayoshi Yamada and Akira Kamikawa, Tokyo Metropolitan Area; Drs. Tadashi Matsuda, Sadayoshi Torigoe, Hitoshi Ochiai, Ritsue Nii, Naoki Yasuda and Takashi Kato, Mie Prefecture; Drs. Koichi Baba and Urara Kohdera, Osaka Prefecture; Drs. Takao Nagai, Takamichi Mukaida, Tetsushi Inoue, Junji Suzue, Fumihiko Hamada, Akira Takehiro, Atsuko Nishioka, Hitoshi Jinnai, Takuji Fujisawa and Kenkichi Sasaki, Shikoku region; and, Drs. Yoshio Takasaki, Shizuo Shindo, Naoki Tsumura and Yuji Yamashita, Fukuoka Prefecture.

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ORIGINAL

Effectiveness of Influenza vaccines in reducing risk of acute febrile illness among community-dwelling elderly, 2006-07 seasons: Population-based cohort study in Japan

Asae OURA[1,2], Hirofumi OHNISHI[2], Megumi HARA[3], Fumio SAKAUCHI[2], Yasuhiro FUSHIKI[2], Mitsuru MORI[2]

[1] Research Fellow of the Japan Society for the Promotion of Science (JSPS Research Fellow),

[2] Department of Public Health, Sapporo Medical University School of Medicine,

[3] Department of Preventive Medicine, Faculty of Medicine, Saga University)

ABSTRACT

Background: Annual vaccinations are recommended for groups with high-risk medical conditions, as well as the residents of nursing homes. In general, little is known about the vaccine effectiveness of vaccines for Influenza-like illness (ILI) in community-dwelling elderly.

Methods: A population based cohort study was conducted during the 2006-2007 (06/07) influenza season to examine the effectiveness of an influenza vaccine among community-dwelling elderly. We selected 1,000 elderly citizens ranging from 65 to 74 years old randomly from a population registry of Sapporo in September 2006. Baseline survey for them was conducted in October or November 2006, and 542 (54.2%) subjects responded with an informed consent. We excluded one person because he passed away before the follow-up survey. Thus, we analyzed 541 subjects. We followed-up the participants concerning acute febrile illnesses, hospitalizations, and so on every prior month from December 2006 to April 2007 though telephone interviews. The Chi-square test and Mann-Whitney U-test were used to compare vaccinated group to non-vaccinated group, and Cox's hazard model was conducted to control for potential confounding factors.

Results: After adjusting for confounders, the vaccination decreased acute fevers higher than or equal to 37.5°C (Hazard ratio (HR) =0.42, 95% confidence interval (CI)=(0.20, 0.90)) from December 2006 to March 2007, but was not associated with the risk of ILI (HR=1.25, 95%CI=(0.29, 5.37)).

Conclusion: An influenza vaccination may decrease an acute fever during an influenza epidemic season in community-dwelling elderly.

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Key words: Influenza vaccine, Effectiveness, Influenza-like illness, Community-dwelling elderly

1 INTRODUCTION

According to the Advisory Committee on Immunization Practices (ACIP) in the United States, inactivated influenza vaccination is 30-70% effective in preventing hospitalization for pneumonia and influenza among elderly persons not living in nursing homes or chronic-care facilities. Annual vaccinations are recommended for those groups, as well as the residents of nursing homes and for those groups with high-risk medical conditions[1]. The Japanese Ministry of Health, Labour and Welfare recommended a vaccination including the assistance though public funds, for a senior citizens older than 65 years old from 2001, because they recognized the elderly to be a high-risk group for influenza.

Most observational studies about the effectiveness of influenza vaccines in community-dwelling elderly have been investigated by linkage to large scale databases[2-10]. Because existing administrative databases cannot be used to evaluate the effectiveness of influenza vaccination in Japan, another approach to evaluate the effectiveness of an influenza vaccination is needed[11]. All subjects should be followed equally thorough the influenza season to examine the effects of the influenza vaccination for

influenza-like illness (ILI) and high fever, as such studies have so far mostly been limited to elderly residents in nursing homes[12,13]. In general, little is known about the vaccine effectiveness for ILI in community-dwelling elderly[14]. In the previous cohort study in Saga, Japan[15], influenza vaccination decreased ILI significantly (OR=0.38; 95%CI=(0.17,0.85)) after adjusting for confounders. This present study was a population-based cohort study, conducted during the 06/07 season to examine the effectiveness of an influenza vaccine among community-dwelling elderly in Sapporo, Japan.

2 SUBJECTS AND METHODS

We selected 1,000 elderly citizens ranging from 65 to 74 years old randomly from a population registry of Sapporo City in September 2006. We sent them a letter with an explanation of the study and requesting for their participation. The eligibility criteria to participate in study were as follows; not being hospitalized, not being institutionalized, and having access to contact by telephone at least once a month. As a baseline survey in October or November 2006, we asked them to answer the self-administered questionnaire about baseline characteristics that might act as potential confounders including: a history of influenza vaccinations (this season(06/07), pre-season(05/06), and the season before last(04/05)), a diagnosis of influenza (this season(06/07), pre-season(05/06), and the season before last(04/05)), health condition by self report, health status (underlying disease etc), history of ILI, vaccination, smoking habits, exercise habits, going out to crowded areas, history of hospitalizations for pneumonia, day care or day service or short stay use, hand washing and gargling habits, and family constitution. Among the 1,000 elderly citizens, 542 (54.2%) subjects responded with a written informed consent. We excluded one person because he passed away before the follow-up survey. Thus, we followed-up and analyzed 541 subjects.

The survey period was defined as from 1 November 2006 to 31 March 2007. We performed a follow-up survey by telephone in December 2006, and in January, February, March, and April 2007 (five times in total). Every month, we interviewed the elderly regarding ILI, acute febrile illnesses, hospitalizations, and death during the previous month by telephone. When an event occurred, we asked the elderly when the event did happen. ILI was defined that community-dwelling elderly have received a diagnosis at a hospital during the epidemic period in this study.

This study was approved by the Ethical Boards of Sapporo Medical University.

3 ANALYSIS

Statistical analyses were performed using the Statistical Package for Social Science (SPSS). The Chi-square test and Mann-Whitney U-test were used to compare vaccinated group and non-vaccinated group. Cox's hazard model was conducted to control for any confounding factors. The HR and their 95%CI were calculated for each factor based on the Cox's hazard model coefficient and standard error. For each of the estimations, the HR was adjusted for gender, age, and underlying disease (one or more of the following criteria; high blood pressure, a cardiovascular disease, a respiratory system disease, diabetes, a cerebrovascular disease). A level of 0.05 was used as the critical level of significance.

4 RESULTS

A total of 541 community-dwelling elderly were followed during the 06/07 influenza season. Participants included 306 (56.6%) males with a mean age (± standard deviation; SD) of 69.5 ±2.9 years. The rate of vaccination was 56.7%. Table 1 shows a comparison of the baseline variables between the vaccinated group and the non-vaccinated group. The vaccinated group was more likely to be female ($p=0.01$), older ($p<0.01$), to have underlying disease ($p<0.01$), to have never been a smoker ($p=0.01$), to have family medicine ($p<0.01$), and to gargle after returning home ($p<0.01$) than the non- vaccinated group.

Table 2 shows the ILI affection, and the vaccination situation in 2005/2006 (05/06) seasons. The vaccinated group in the 05/06 season was less likely to have an ILI in the 05/06 season ($p<0.01$), and more likely to vaccinate in the 06/07 season ($p<0.01$) than the non-vaccinated group in 05/06.

Table 3 illustrates the ILI affection and the vaccination situation in the 04/05 seasons. The vaccinated group in the 04/05 season was less likely to have an ILI in the 04/05 season ($p=0.04$), and more likely to vaccinate in the 06/07 season ($p<0.01$) than their counterparts.

The effect of the vaccine for each event is shown in Table 4. When the patients vaccinated, the risk of fever higher than or equal to 37.5°C for the latter was reduced compared with their counterparts (crude: HR=0.47, 95%CI=(0.23, 0.97); adjusted: HR=0.42, 95%CI=(0.20, 0.90)). We compared the groups with and without underlying diseases, and the factor of having an

Table 1 Baseline characteristic

	The vaccinated group (n=307)	The non-vaccinated group (n=234)	p-value$
Gender (Male)	159 (51.8%)	147 (62.8%)	0.01
Age (years old)	70.0±2.8	68.8±2.9	<0.01
Having underlying disease (yes)*	189 (61.6%)	107 (45.7%)	<0.01
Health condition (good, normal)	281 (91.5%)	218 (93.2%)	0.52
Smoking habits (yes)	39 (12.7%)	50 (21.4%)	0.01
Regular exercise (More than once a week)	195 (63.5%)	146 (62.4%)	0.79
Having family medicine (yes)	259 (84.4%)	154 (65.8%)	<0.01
Going out to crowd areas (more than once a week)	253 (82.4%)	186 (79.5%)	0.44
Number of family members living together	2.7±1.4	2.6±1.1	0.31
Living together with a kindergarten, nursery, or primary schoolchild (yes)	23 (7.5%)	11 (4.7%)	0.21
Washes hands after returning home (yes)	279 (90.9%)	209 (89.3%)	0.57
Gargles after returning home (yes)	256 (83.4%)	169 (72.2%)	<0.01
Using a day-care or day-service (More than once a week)	7 (2.3%)	3 (1.3%)	0.53
Using a short stay service (yes)	1 (0.3%)	2 (0.9%)	0.58

number (%), means ± SD

underlying disease *: one or more of the following criteria: high blood pressure, a cardiovascular disease, a respiratory system disease, diabetes, a cerebrovascular disease

p-value$: Fisher's exact test, Mann-Whitney's U test.

Table 2 Influenza-like illness (ILI) affection in the 05/06 seasons, and the vaccination situation in the 06/07 seasons; comparing the vaccinated group and non-vaccinated group in the 05/06 season

	The vaccinated group in the 05/06 season (n=282)	The non-vaccinated group in the 05/06 season (n=259)	p-value$
ILI in the 05/06 season	3 (1.1%)	13 (5.0%)	<0.01
Vaccination in the 06/07season	263 (93.3%)	44 (17.0%)	<0.01

number (%)

p-value$: Fisher's exact test

Table 3 Influenza-like illness (ILI) affection in the 04/05 seasons, and the vaccination situation in the 06/07 seasons; compared vaccinated group and non-vaccinated group in the 04/05 season

	The vaccinated group in the 04/05 season (n=231)	The non-vaccinated group in the 04/05 season (n=310)	p-value$
ILI in the 04/05 season	4 (1.7%)	16 (5.2%)	0.04
Vaccinated in the 06/07season	208 (90.0%)	99 (31.9%)	<0.01

number (%)

p-value$: Fisher's exact test

underlying disease was not significant, although we did not show the data in the table.

Table 5 demonstrates the baseline characteristic in fevers higher than or equal to 37.5°C. The group that had a fever higher than or equal to 37.5°C were more likely to live together with a daughter (p=0.01), or kindergarten, nursery, or primary schoolchild (p=0.04) than the group that didn't have a fever higher than or equal to 37.5°C. Also, the group that had a fever higher than or equal to 37.5°C were less likely to vaccinate in 05/06 (p=0.03) than their counterparts.

Table 4 Effect of the vaccine for each event

	The vaccinated group (n=307)	The non-vaccinated group (n=234)	Hazard ratio* (95%CI[¤])	Hazard ratio** (95%CI[¤])
Fever (≧37.0°C)	27 (8.8%)	27 (11.5%)	0.75 (0.44, 1.28)	0.74 (0.42, 1.29)
(≧37.5°C)	12 (3.9%)	19 (8.1%)	0.47 (0.23, 0.97)	0.42 (0.20, 0.90)
(≧38.0°C)	10 (3.3%)	14 (6.0%)	0.54 (0.24, 1.21)	0.48 (0.21, 1.12)
ILI	4 (1.3%)	4 (1.7%)	0.75 (0.19, 3.01)	1.25 (0.29, 5.37)
Pneumonia	2 (0.7%)	0 (0%)	- †	- †
Hospitalization for influenza	2 (0.7%)	0 (0%)	- †	- †

number (%)

p-value[§]: Fisher's exact test

CI[¤]: confidence interval

The vaccinated group vs. the non-vaccinated group

Hazard ratio*: crude

Hazard ratio**: adjusted for sex, age, and underlying disease (one or more of the following criteria; high blood pressure, a cardiovascular diease, a respiratory system disease, diabetes, a cerebrovascular disease).

† : Could not be calculated.

Table 5 Baseline characteristic in fever higher than or equal to 37.5°C

	More higher than or equal to 37.5°C		p-value[§]
	Not having (n=510)	Not having (n=510)	
Gender (Male)	15 (48.4%)	291 (57.1%)	0.36
Age (years old)	69.2±3.1	69.5±2.9	0.58
Having underlying disease* (yes)	18 (58.1%)	278 (54.5%)	0.85
Health condition (good, normal)	27 (87.1%)	472 (92.5%)	0.29
Smoking habits (yes)	3 (9.7%)	86 (16.9%)	0.45
Regular exercise (More than once a week)	17 (54.8%)	324 (63.5%)	0.34
Going out to crowded areas (more than once a week)	25 (80.6%)	414 (81.2%)	1.00
Number of family members living together	3.1±1.9	2.6±1.2	0.14
Living together with a daughter (including the justice) (yes)	13 (41.9%)	109 (21.4%)	0.01
Living together with a kindergarten, nursery, or primary schoolchild (yes)	5 (16.1%)	29 (5.7%)	0.04
Washes hands after returning home (yes)	29 (93.5%)	459 (90.0%)	0.76
Gargles after returning home (yes)	22 (71.0%)	403 (79.0%)	0.27
Using a day-care or day-service (More than once a week)	0 (0%)	10 (2.0%)	- **
Using a short stay service (yes)	0 (0%)	3 (0.6%)	- **
Vaccinated in the 05/06 (yes)	10 (32.3%)	272 (53.3%)	0.03
Vaccinated in the 04/05 (yes)	10 (32.3%)	221 (43.3%)	0.27

number (%), means ± SD

underlying disease *: one or more of the following criteria: high blood pressure, a cardiovascular disease, a respiratory system disease, diabetes, a cerebrovascular disease

p-value[§]: Fisher's exact test, Mann-Whitney's U test.

**: Could not be calculated.

5 DISCUSSION

In the baseline characteristics, the vaccinated group was more likely to never have been a smoker, and gargle after returning home. There is possibility that the vaccinated group had a healthier consciousness than their counterparts, and there may have also been selection bias. The vaccinated group may have taken the recommendation of the vaccination more than the non-vaccinated group, because the vaccinated group was more likely to have an underling disease and family medicine.

In the present study, the vaccinated group in the 05/06 season was less likely to have an ILI in the 05/06 season (the vaccinated vs. the non-vaccinated: 1.1% vs. 5.0%) and the vaccinated group in the 04/05 season was less likely to have an ILI in the 04/05 season (the

vaccinated vs. the non-vaccinated: 1.7% vs. 5.2%) than their counterparts. Hara et al[16] reported vaccinated group were less likely to have ILI than non-vaccinated group. The result of the present study supported the result of the study[16], though year in an influenza season, the type of endemic influenza viruses, and area in Japan were different. However, there is a possibility of misclassification[11] or selection bias, because the vaccinated group may be likely to attend a hospital after they become sick. This study of effectiveness of an influenza vaccine in persons aged 65 years or over living in a community was limited[17-19], especially in the effectiveness for ILI[14, 20].

The vaccinated group in the 05/06 season was more likely to vaccinate in the 06/07 season than the non-vaccinated group in the 05/06 (the vaccinated vs. the non-vaccinated: 93.3% vs. 17.0%), and the vaccinated group in the 04/05 season was more likely to vaccinate in the 06/07 season (the vaccinated vs. the non-vaccinated: 90.0% vs. 31.9%) than their counterparts. If the community-dwelling elderly were vaccinated once, the elderly may be vaccinated again the next year. Therefore, it would be important for the Japanese Government to intervene to find ways to vaccinate for the elderly.

In this present cohort study among community-dwelling elderly, influenza vaccination reduced the risk of a fever higher than or equal to 37.5°C during the epidemic period. The result of the present study supported the result of the previous cohort study in Saga[15]. Critical point of high fever of this study (37.5°C) was different from that of the previous study in Saga (38.5°C)[16]. Because there are no other reports, to our knowledge, regarding effectiveness of influenza vaccine reducing risk of acute febrile illness, future study is necessary to identify the critical point of high fever for vaccine effectiveness. In a follow-up survey over the telephone, it was difficult to perform an influenza judgment in the present study because the survey asked a self-report, however it is correct for judging the fever. A high fever from influenza is an important health hazard in elderly in a community.

Comparison between a high-risk condition group and a low-risk condition group are important in a general influenza study[21], and it was not significant in the present study. Although we compared risk of having ILI between a high-risk group and a low-risk group, it was not significantly different. No difference between them might occur from small sample size, relatively low response rate (54%), or low prevalence of pneumonia during this influenza seasons in Japan compared with a report in Europe[22]. Such variables do not capture the important difference in health status between vaccinated and unvaccinated individuals, and the adjustment for these variables alone does not remove the confounding factors. In our future study, we will control for confounding with detailed medical record information.

In the present study, the group that had a fever higher than or equal to 37.5°C was more likely to live together a daughter (p=0.01), or a kindergarten or nursery or a primary schoolchild (p=0.04) than the group that didn't have a fever higher than or equal to 37.5°C. There is possibility that a child brought the influenza virus into the house. ACIP[1] has recommended that health-care workers take vaccination against seasonal influenza. It is thought that prevention in the whole family is important.

Certain limitations in the present study should be disclosed. First, the present study may have had a selection bias because the response rate was 54.2%. Second, the number of subjects was insufficient. Third, there was the possibility of misclassifications in the present study because the judgment of influenza was taken via a self-report, although we confined it to physician's diagnosed ILI.

In conclusion, a population based cohort study was conducted during the 06/07 influenza season to examine the effectiveness of an influenza vaccine among 541 community-dwelling elderly, ranging from 65 to 74 years. After adjusting for confounders, the vaccination decreased acute fevers higher than or equal to 37.5°C (HR =0.42, 95% CI=(0.20, 0.90)) during the epidemic period. Therefore influenza vaccinations may be decreased acute fevers during influenza epidemic periods in community-dwelling elderly.

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Correspondence:
Asae OURA
Department of Public Health, School of Medicine, Sapporo Medical University
Chuo-ku Minami-1 Nishi-17, Sapporo, Hokkaido, 060-8556, Japan
phone number: +81-(0)11-611-2111
FAX number: +81-(0)11-641-8101
Email: aoura@sapmed.ac.jp