Effectiveness of the 2019–2020 influenza vaccine and the effect of prior influenza infection and vaccination in children during the first influenza season overlapping with the COVID-19 epidemic

Running title: Influenza vaccine effectiveness

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Abstract

Background: The behavioral changes among Japanese, along with the coronavirus disease 2019 (COVID-19) epidemic, may affect the seasonal influenza epidemic in Japan and change the influenza vaccine effectiveness (VE).

Methods: Influenza VE in children was estimated in the first influenza season (2019/20) overlapping with the COVID-19 epidemic by conducting a single-center, test-negative case-control (TNCC) study. Effects of prior influenza infection and vaccination in children were assessed for the 2019–2020 season.

Results: Among 386 children, the adjusted VE was significant for influenza A/H1N1 (45.5%; 95% confidence interval (CI): 2.0–69.7) and influenza B (66.7%; 95% CI: 35.9–82.7). Among patients aged 0–6 years, the adjusted VE was significant for influenza A (total: A/H1N1+A/H3N2) (65.0%; 95% CI: 22.2–84.3), influenza A/H1N1 (64.8%; 95% CI: 16.9–85.1), and influenza B (87.4%; 95% CI: 50.5–96.8). No VE was observed in patients aged 7–15 years. Two vaccine doses tended to decrease the incidences of influenza A (total) and influenza A/H1N1 in patients aged 0–6 years. The adjusted odds ratios (ORs) of influenza B infection in patients, who had influenza during the previous season, were significantly low among all participants (0.29; 95% CI: 0.11–0.78) and patients aged 7–15 years (0.34; 95% CI: 0.12–0.94). The adjusted ORs of influenza infections were not significant in patients vaccinated during the previous season.

Conclusions: TNCC-based estimates of influenza VE were consistent despite the overlapping COVID-19 epidemic.

Key words: influenza vaccine effectiveness, single-center study, test-negative case-control study
Introduction

The test-negative case–control (TNCC) study is a validated approach for evaluating vaccine effectiveness (VE) against influenza \(^1\)-\(^3\). Multiple reports published in Japan provide estimates for the efficacy of quadrivalent influenza vaccines using TNCC studies \(^4\)-\(^10\). During the 2019–2020 influenza season, the outbreak of coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) started in Wuhan, China, in December 2019 \(^11\). In Japan, the first case was reported on January 16, 2020, followed by the gradual spread of the COVID-19 epidemic throughout the country \(^12\). The Japanese government recommended that people should exercise self-restraint as a social distancing measure, which led to a clear decline in both going out and having person-to-person contact. Moreover, people were regularly reminded to wear a mask and wash the hands frequently. The behavioral changes among Japanese individuals, along with the COVID-19 epidemic, may affect the seasonal influenza epidemic in Japan and change the influenza vaccine effectiveness (VE). Furthermore, although the effect of prior infection and vaccinations on the seasonal influenza VE has been examined in recent years \(^13\)-\(^15\), it has not been assessed in the context of the specific circumstances of the 2019–2020 influenza season. Here, I designed a TNCC study to estimate the effectiveness of the quadrivalent influenza vaccine formulation in children during the 2019–2020 season and to examine the potential effect of prior infection and vaccination on the influenza VE in the current season. The results will validate the TNCC study design and show the effect of prior influenza infection and vaccination on influenza VE during the recent season overlapping with the COVID-19 epidemic.

Materials and methods
Patients and data collection

The participants were children who were subjected to the rapid influenza diagnostic test (RIDT) in the Ando Clinic (Narashino City, Chiba, Japan) due to suspected influenza infection during the 2019–2020 season. The pediatric patients, their parents, or both were informed of the concept of this study. Patients who fulfilled the criteria of influenza-like illness (ILI) were included. The ILI diagnosis was based on the definition issued by the World Health Organization (WHO): “Patients in whom influenza infection was suspected, evidenced by symptoms including acute onset, nasal discharge, sore throat, cough, arthralgia, and myalgia”\textsuperscript{16}. The interval from the time of administration of quadrivalent inactivated influenza vaccination to the diagnosis of ILI was ≥14 days and <5 months\textsuperscript{17}. The following patients were excluded: patients who had already experienced the same type of influenza infection during the 2019–2020 season; patients who had already received a neuraminidase inhibitor due to negative results of the RIDT.

The patients were divided into two age groups: 0–6 and 7–15 years. The following clinical information was collected: sex, age, the period of influenza infection onset, vaccination status for the quadrivalent influenza vaccine, comorbidities, influenza infection during the previous season, and vaccination status from the previous season at presentation.

Comorbidities were defined as the following conditions that may affect the immune status: chronic pulmonary, cardiovascular (excluding hypertension), renal, hepatic, hematologic, and neurological disorders, diabetes mellitus, autoimmune disorders, congenital anomaly, and cancer.

Influenza diagnosis
Nasopharyngeal swabs were obtained and tested using ImunoAce™ Flu and Linjudge™ FluA/pdm (TAUNS Laboratories, Inc., Shizuoka, Japan). As previously reported, influenza A (H1N1) pdm09 was diagnosed based on both ImunoAce™ Flu, which detected positivity for influenza A, and Linjudge™ FluA/pdm, which also detected positivity. Influenza A (H3N2) was diagnosed when the ImunoAce™ Flu test was positive for influenza A and the Linjudge™ FluA/pdm test was negative.

**Vaccine**

The quadrivalent influenza vaccine contained influenza A/Brisbane/02/2018(IVR-190) (H1N1) pdm09 strain, A/Kansas/14/2017 (X-327) (H3N2) strain, B/Phuket/3073/2013 strain (Yamagata lineage), and influenza B/Maryland/15/2016 (NYMC BX-69A) strain (Victoria lineage). At 2–4-week intervals, two 0.25 mL and 0.5 mL doses of vaccine were administered to children aged 6 months to 2 years and 3–12 years, respectively. A single 0.5 mL vaccine dose was generally administered to children aged ≥13 years.

**VE and TNCC study**

As previously reported, VE was estimated using a TNCC study design. VE was defined as 
\[ \{1 - \text{odds ratio (OR)}\} \times 100, \]
and OR was calculated as 
\[ \frac{\text{number of influenza-positive among vaccinated patients}}{\text{number of influenza-negative among unvaccinated patients}} \times \frac{\text{influenza-negative among vaccinated patients}}{\text{influenza-positive among unvaccinated patients}}. \]

OR values were assessed using Wald test. First, the crude VE was calculated and adjusted for sex, age group (0–6 vs. 7–15 years), the period of influenza infection onset (December 2019–January 2020 vs. February–March 2020), and comorbidity. When the VE assessment was based on the number of vaccine doses in children, a multivariate logistic regression analysis was performed using the age, body temperature, time from onset, and
vaccine doses as covariables.\(^9,10\)

**Effect of influenza infection and vaccination status of the previous season**

Based on a previous report\(^15\), I compared the OR of influenza infection of the current season for patients irrespective of whether they had an influenza infection or influenza vaccination during the previous season. Patients with unknown status of influenza infection or vaccination were excluded. In addition, patients less than 1 year of age (i.e., patients who were not born in the previous year) were excluded. Crude OR values were calculated according to the TNCC study design. Thereafter, the OR values were adjusted for sex, age group (0–6 vs. 7–15 years), the period of influenza infection onset (December 2019–January 2020 vs. February–March 2020), and influenza vaccination in all patients during the current season. In each age group, the OR values were adjusted for sex, the period of influenza infection onset (December 2019–January 2020 vs. February–March 2020), and influenza vaccination during the current season.

**Statistical analysis**

Mann–Whitney U test was used to compare continuous variables (i.e., age, body temperature, and time from onset) between RIDT-positive (case) and RIDT-negative (control) subjects. Fischer’s exact tests were used to compare nominal variables (i.e., sex, age group, onset period, and comorbidities). The VE was adjusted for sex, age group, the period of influenza infection onset, and presence/absence of comorbidities.\(^4-10\) Results with two-sided \(P\) values of <0.05 were considered significant. Statistical analyses were performed using JMP® 15.2 (Statistical Analysis Software, SAS Institute Inc., Cary, NC, USA).

**Ethics**
This study was conducted in accordance with The Code of Ethics of the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from the patients, their parents, or both. Participants were recruited prospectively. The study design was approved by the Joint Institutional Review Board (approval number: 14000050.20191220-4830).

Results

Enrollment and trend of influenza infection during the 2019–2020 season

From December 21, 2019 to March 31, 2020, 386 patients were enrolled. In total, 386 patients and 386 episodes were analyzed (none of the patients had an episode of influenza A and B). The trend of influenza A infection started in December 2019 and peaked in January 2020 (Fig. 1); then, the influenza A epidemic declined and disappeared by March 2020. Among the total influenza A infections, the proportion of influenza A/H1N1 was substantially higher than that of influenza A/H3N2. The influenza B epidemic started in January 2020, and it peaked and overtook the influenza A epidemic in February 2020 (Fig. 1). The influenza B epidemic declined in March 2020.

Patient characteristics

Patient characteristics are summarized in Table 1. In total, 136 episodes were RIDT-positive (case) and 250 were RIDT-negative (control). RIDT-positive patients were significantly older than RIDT-negative patients (mean age ± standard error; 8.2 ± 0.3 vs. 6.4 ± 0.2; \( P < 0.0001 \)). The proportion of cases without influenza vaccination during the previous season was significantly smaller among the RIDT-positive cases than among the RIDT-negative cases (presence:absence of influenza vaccination during the previous season: 57:76 vs. 134:103, respectively; \( P = 0.0128 \)). The body temperature of RIDT-positive patients was significantly
higher than that of RIDT-negative patients (mean body temperature [°C] ± standard error: 39.2 ± 0.1 vs. 38.8 ± 0.0, respectively; \( P < 0.0001 \)). The time from influenza infection onset was significantly longer for RIDT-positive patients than for RIDT-negative patients (mean time from onset [h] ± standard error: 22.7 ± 1.3 vs. 17.7 ± 1.0, respectively; \( P = 0.0002 \)).

Comorbidities as follows: 21 patients = bronchial asthma, 1 = epilepsy, 1 = Kawasaki disease, and 1 = hereditary spherocytosis.

**VE assessment**

Table 2 presents the VE analysis results. Among all patients, the adjusted VE was significant for influenza A/H1N1 (45.5%; 95% confidence interval (CI): 2.0–69.7) and influenza B (66.7%; 95% CI: 35.9–82.7). In the age group of 0–6 years, the adjusted VE was significant for the influenza A (total), A/H1N1+A/H3N2 (65.0%; 95% CI: 22.2–84.3), influenza A/H1N1 (64.8%; 95% CI: 16.9–85.1), and influenza B (87.4%; 95% CI: 50.5–96.8). There was no significant VE in the age group of 7–15 years.

**Vaccine doses**

The correlation between vaccine doses and the adjusted ORs of incidence of influenza is shown in Table 3. In the age group of 0–6 years, two vaccine doses tended to decrease the incidence of influenza A (total) and influenza A/H1N1 compared with the incidence among those without vaccination or only one dose. Specifically, in the age group of 0–6 years, the adjusted OR was significant for influenza A (total): 0.58 (95% CI: 0.37–0.88) in cases with one dose, and 0.33 (95% CI: 0.14–0.78) with two doses (\( P = 0.0116 \)). In the same age group, the adjusted OR values against influenza A/H1N1 were 0.58 (95% CI: 0.37–0.93) in cases with one dose and 0.34 (95% CI: 0.14–0.86) with two doses (\( P = 0.0231 \)). A dose-
dependent response relationship was suggested for the VE in the age group of 0–6 years. However, regardless of the dosing regimen, the adjusted VE was not significant in the age group of 7–15 years (Table 3).

**Effect of influenza infection during the previous season**

The ORs of influenza infection for the current season in relation to influenza infection during the previous season are shown in Table 4. Among all subjects, the adjusted OR was significant for influenza B (0.29; 95% CI: 0.11–0.78; \( P = 0.0140 \)). The age group of 0–6 years had no significant ORs associated with influenza A (total) and influenza A/H1N1, and ORs could not be calculated for influenza A/H3N2 and influenza B. In the age group of 7–15 years, the adjusted OR was significant for influenza B (0.34; 95% CI: 0.12–0.94; \( P = 0.0368 \)). Furthermore, the adjusted OR tended to be lower for influenza A (total) (0.43; 95% CI: 0.16–1.14; \( P = 0.0890 \)) and influenza A/H1N1 (0.39; 95% CI: 0.13–1.15; \( P = 0.0878 \)) in the age group of 7–15 years, but none of the adjusted ORs was significant (Table 4).

**Effect of influenza vaccination status during the previous season**

The ORs of influenza infection for the current season in relation to influenza vaccination during the previous season are shown in Table 5. Neither the influenza types nor the two age groups were associated with a significant OR (Table 5).

**Discussion**

The 2019–2020 influenza season was a peculiar season because of the COVID-19 epidemic. Most Japanese were forced to refrain from going out and having person-to-person contacts, and they were regularly reminded to wear a mask and to wash their hands frequently. Japanese initiatives for public health differed substantially from previous influenza seasons. The COVID-19 epidemic, along with the changes in the behavior of
Japanese individuals, might have affected the seasonal influenza epidemic and the influenza VE. In fact, during the previous season in my clinic, approximately twice as many subjects were enrolled, than the number of participants during the current season\textsuperscript{9,10}. The COVID-19 epidemic spread more widely abroad than in Japan\textsuperscript{18}. In Europe, the interim VE estimate against influenza A/H1N1 was not significant among children in primary care settings\textsuperscript{19}. In the United States, the interim VE estimate against influenza A/H1N1 was significant in older adults (\geq 50 years old), but it was not significant against influenza A/H1N1 in younger adults (aged 19–49 years)\textsuperscript{20}. These results differed slightly from the prior season\textsuperscript{21,22}. However, the results of this study were comparable with those of previous studies\textsuperscript{9,10}. This can be attributed to the following reasons. First, the TNCC study design may be firm despite the small number of patients. Second, the influenza epidemics ended before the COVID-19 epidemic seriously influenced the influenza epidemics. In the present study, including all participants, the VE was significant for influenza A/H1N1 (45.5\%) and influenza B (66.7\%) in children. This result was comparable or slightly inferior to that reported in the United States (influenza A/H1N1: 51\%; 95\% Cl: 22–69; influenza B: 56\%; 95\% Cl: 42–67)\textsuperscript{20} and Canada (influenza A/H1N1: 63\%; 95\% Cl: 25–81; influenza B: 77\%; 95\% Cl: 59–87)\textsuperscript{23}. Furthermore, the trends in the subdivided age groups of the present study were similar to those observed in my previous studies\textsuperscript{9,10}. Although the quadrivalent influenza vaccine showed significant VE against influenza A (total), influenza A/H1N1, and influenza B among pediatric patients aged 0–6 years, there was no substantial VE among children aged 7–15 years. The VE of the quadrivalent influenza vaccine against influenza A (total) and influenza A/H1N1 appeared to be dose-dependent among children aged 0–6 years. The reason for this substantial difference in VE
between younger and older children remains unclear. Sugaya et al. speculated that low VE in older children (13–15 years old) could be owing to the immunological effects of repeated vaccination, as opportunities for influenza vaccination and/or influenza infection increase with age. In contrast, younger children are immunologically naïve. However, my study showed that the adjusted OR of the current influenza B epidemic was significantly lower among patients aged 7–15 years who had an influenza infection during the previous season (0.34; 95% CI: 0.12–0.94; P = 0.0368). Furthermore, although the adjusted OR was not significant, it tended to be lower for influenza A (total) (0.43; 95% CI: 0.16–1.14; P = 0.0890) and influenza A/H1N1 (0.39; 95% CI: 0.13–1.15; P = 0.0878) among patients aged 7–15 years who had influenza infection during the previous season. However, the influenza infection status during the previous season had no effect on patients aged 0–6 years old during the current season. Thus, several factors from the previous season, besides vaccination, may affect the influenza VE in older children, compared with that in younger children.

Moreover, there were no significant ORs of influenza infection in the current season in relation to the influenza vaccination during the previous season among all age groups. This result was similar to that of previous studies, indicating the importance of the current vaccination.

This study had some limitations. First, the bias due to the small sample size may have affected the results of this TNCC study. However, the present study lays a solid basis for future research using a larger number of patients. Furthermore, the results of this study were highly similar to those of my studies in previous influenza seasons, as well as studies in the United States and Europe during the 2019–2020 season. Second, sampling bias was
unavoidable because this study was conducted in a single clinic. However, as the patients were treated by the same physician, medical practice was constant across all cases. Third, as 2.9% of the positive cases detected by Linjudge™ FluA/pdm presented a weak positive reaction for influenza A/H3N2 (information provided on the package insert), the diagnosis of influenza A/H1N1 pdm09 may not always be accurate. However, the diagnosis of influenza A/H1N1 pdm09 was only made when Linjudge™ FluA/pdm showed a clear positive reaction, according to my previous study. Fourth, the VE against influenza A/H3N2 pdm09 could not be confirmed because this study had a relatively small number of influenza A/H3N2 cases. However, the paucity of influenza A/H3N2 was a trend of this season in Japan. Fifth, due to the small number of participants, I was unable to subdivide the patient population into multiple groups, as in an earlier study on ORs of seasonal influenza cases in relation to prior influenza infection and vaccination. However, the results of the present study were comparable with those of previous studies. Finally, RIDT is not completely accurate. The influenza virus was not precisely identified here, such as by virus isolation and RT-PCR. A previous study did not find significant differences between RT-PCR data and the results estimated by the RIDT using a brand different from that used in the present study. Furthermore, the RIDT of this study had a comparable or higher concordance rate with viral isolation cultures than that of previous studies. Thus, although a confirmation study should still be conducted, the results of this study might be equivalent to a study using RT-PCR.

In conclusion, this is the first study to assess the VE of the inactivated quadrivalent influenza vaccine in children during the 2019–2020 influenza season, which was the first influenza season that overlapped with the COVID-19 epidemic in Japan. In patients aged
0–6 years, the VE was significant against influenza A (total), influenza A/H1N1, and influenza B. This season’s VE was comparable with that of previous years, and it appears that the TNCC study generated robust results during the 2019–2020 season. Although no significant VE was observed in the age group of 7–15 years, there was a significantly lower OR for influenza B with influenza infection during the previous season. Besides prior influenza vaccination, multiple factors from previous influenza seasons may affect the 2019–2020 influenza VE in older children. Interestingly, the influenza vaccination of the current season appeared to have a beneficial effect rather than that of the previous season, which indicated the importance of having children vaccinated every year. As there was a significant influenza VE in younger children aged 0–6, the annual vaccination of this age group is recommended for the 2020–2021 season. Moreover, the annual vaccination of older children without influenza infection during the 2019–2020 season is recommended.

Conflicts of interest
The author has no potential conflict of interest to disclose.

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**Figure legend**

Fig. 1 Proportion of infections caused by influenza A/H1N1, influenza A/H3N2, and influenza B among children during the 2019–2020 influenza season.
Fig. 1

![Graph showing the number of patients infected with different influenza strains from December 2019 to March 2020. The x-axis represents the months, and the y-axis represents the number of patients. The bars indicate the number of patients infected with Influenza A/N1N1, Influenza A/H3N2, Influenza B, and Negative.]

- **Influenza A/N1N1**
- **Influenza A/H3N2**
- **Influenza B**
- **Negative**
Table 1 Patient characteristics

| Characteristic                                      | Total  | Test-positive (case) | Test-negative (control) | P-value |
|-----------------------------------------------------|--------|----------------------|-------------------------|---------|
| Number (n)                                          | 386    | 136                  | 250                     | -       |
| Age (mean age)                                      | 7.0 ± 0.2 | 8.2 ± 0.3          | 6.4 ± 0.2               | <0.0001 * |
| Sex (male:female)                                   | 211:175 | 78:58                | 133:117                 | 0.4551  |
| Onset period (first half:second half) b             | 224:162 | 80:56                | 144:106                 | 0.8299  |
| Vaccination (yes:no)                                | 213:173 | 58:78                | 155:95                  | 0.0004 * |
| Comorbidity c (yes:no)                              | 24:362  | 13:123               | 11:239                  | 0.0500  |
| Influenza infection during previous season (yes:no) d| 70:301  | 18:115               | 52:186                  | 0.0535  |
| Influenza vaccination during previous season (yes:no) e| 191:179 | 57:76                | 134:103                 | 0.0128 * |
| Body Temperature (°C)                               | 38.9 ± 0.0 | 39.2 ± 0.1          | 38.8 ± 0.0              | <0.0001 * |
| Time from onset e (hours)                           | 19.4 ± 0.8 | 22.7 ± 1.3          | 17.7 ± 1.0              | 0.0002 * |

Age, body temperature, time from onset: mean ± standard error

a Types of influenza were as follows: 64, influenza A/H1N1; 14, influenza A/H3N2; 58, influenza B.

b First half: December 2019–January 2020, second half: February–March 2020

c Comorbidities included the following: 21, bronchial asthma; 1, epilepsy; 1, Kawasaki disease; 1, hereditary spherocytosis.
d Fifteen patients were excluded (13: Not born in the previous season; 2: Unknown status of influenza infection in the previous season).

e Sixteen patients were excluded (13: Not born in the previous season; 3: Unknown status of influenza vaccination in the previous season).
|                          | Test-positive | Test-negative | Crude VE (95% CI) | Adjusted VE \(^c\) (95% CI) |
|--------------------------|---------------|---------------|-------------------|-----------------------------|
| **Overall**              |               |               |                   |                             |
| Influenza A (total) \(^a\) | 78 (36/42)    | 250 (155/95)  | 47.5% (12.2 to 68.6) * | 38.5% (-5.6 to 64.2)       |
| Influenza A/H1N1         | 64 (28/36)    | 250 (155/95)  | 52.3% (16.9 to 72.7) * | 45.5% (2.0 to 69.7) *      |
| Influenza A/H3N2         | 14 (8/6)      | 250 (155/95)  | 18.3% (-142.8 to 72.5) | -8.0% (-230.8 to 64.7)    |
| Influenza B              | 58 (22/36)    | 250 (155/95)  | 62.5% (32.5 to 79.2) * | 66.7% (35.9 to 82.7) *     |
| **Age 0–6 years \(^b\)**|               |               |                   |                             |
| Influenza A (total) \(^a\) | 34 (13/21)    | 148 (99/49)   | 69.4% (33.7 to 85.8) * | 65.0% (22.2 to 84.3) *     |
| Influenza A/H1N1         | 28 (11/17)    | 148 (99/49)   | 68.0% (26.4 to 86.1) * | 64.8% (16.9 to 85.1) *     |
| Influenza | Total | Vaccine Effectiveness (95% CI) |
|-----------|-------|-------------------------------|
| A/H3N2    | 6 (2/4) | 148 (99/49) | 75.3% (-39.8 to 95.6) | 75.6% (-38.9 to 95.7) |
| B         | 13 (3/10) | 148 (99/49) | 85.2% (43.6 to 96.1) * | 87.4% (50.5 to 96.8) * |

**Age 7–15 years**

| Influenza   | Total | Vaccine Effectiveness (95% CI) |
|-------------|-------|-------------------------------|
| A (total)   | 44 (23/21) | 102 (56/46) | 10.0% (-82.8 to 55.7) | -5.3% (-124.3 to 50.6) |
| A/H1N1      | 36 (17/19) | 102 (56/46) | 26.5% (-57.4 to 65.7) | 17.8% (-86.6 to 63.8) |
| A/H3N2      | 8 (6/2) | 102 (56/46) | -146.4% (-1179.5 to 52.5) | -216.9% (-1620.8 to 41.6) |
| B          | 45 (19/26) | 102 (56/46) | 40.0% (-21.9 to 70.4) | 53.3% (-2.1 to 78.6) |

VE: vaccine effectiveness, CI: confidence interval

* statistically significant

*a* Influenza A (total) includes influenza A/H1N1 and A/H3N2.

*b* Eleven patients aged 6–11 months were included.

*c* VE adjusted for age group (0–6 vs. 7–15 years), sex, period of influenza infection onset, and comorbidities among all children. VE
adjusted for sex, period of influenza infection onset, and comorbidities in each age group. In the analysis of influenza A/H3N2, comorbidities were excluded from variables. In the analysis of influenza A/H3N2 by age group of 0–6 years, comorbidities and period of influenza infection onset were excluded from variables.
Table 3 Vaccine effectiveness according to vaccine doses

| Vaccine Dose | None       | Once       | Twice       | P-value |
|--------------|------------|------------|-------------|---------|
| **Total child** |            |            |             |         |
| Influenza A (total) | 1.0        | 0.80 (0.58–1.10) | 0.63 (0.33–1.20) | 0.1635 |
| Influenza A/H1N1 | 1.0        | 0.78 (0.55–1.10) | 0.60 (0.30–1.21) | 0.1537 |
| Influenza A/H3N2 | 1.0        | 0.97 (0.50–1.87) | 0.94 (0.25–3.50) | 0.9290 |
| Influenza B     | 1.0        | 0.75 (0.52–1.09) | 0.56 (0.27–1.18) | 0.1280 |
| **Age 0–6 years** |            |            |             |         |
| Influenza A (total) | 1.0        | 0.58 (0.37–0.88) | 0.33 (0.14–0.78) | 0.0116 * |
| Influenza A/H1N1 | 1.0        | 0.58 (0.37–0.93) | 0.34 (0.14–0.86) | 0.0231 * |
Influenza A/H3N2 | 1.0 | 0.55 (0.21–1.41) | 0.30 (0.04–1.98) | 0.2111
---|---|---|---|---
Influenza B | 1.0 | 0.57 (0.25–1.26) | 0.32 (0.06–1.59) | 0.1644

**Age 7-15 years**

| Influenza A (total) | 1.0 | 1.27 (0.74–2.17) | 1.61 (0.55–4.70) | 0.3824 |
|---------------------|-----|------------------|------------------|-------|
| Influenza A/H1N1    | 1.0 | 1.22 (0.67–2.19) | 1.48 (0.46–4.79) | 0.5157 |
| Influenza A/H3N2    | 1.0 | 1.74 (0.65–4.66) | 3.03 (0.42–21.69) | 0.2703 |
| Influenza B         | 1.0 | 0.78 (0.50–1.22) | 0.61 (0.25–1.49) | 0.2780 |

CI: confidence interval

* statistically significant

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*a* Adjusted for age (years), body temperature (°C), and time from onset (h).

*b* Influenza A (total) includes influenza A/H1N1 and A/H3N2.

*c* Eleven patients aged 6–11 months were included.
Table 4 Odds ratio of influenza infection during the 2019–2020 season in relation to the presence or absence of influenza infection during the previous season

| Influenza infection during previous season | Test-positive (Yes/No) | Test-negative (Yes/No) | Crude odds ratio (95% CI) | Adjusted odds ratio \(^d\) (95% CI) | \(P\)-value \(^e\) |
|------------------------------------------|------------------------|------------------------|--------------------------|----------------------------------|------------------|
| Overall                                  |                        |                        |                          |                                  |                  |
| Influenza A (total)\(^a\)                | 75 (12/63)             | 238 (52/186)           | 0.68 (0.34–1.36)         | 0.60 (0.29–1.24)                 | 0.1653           |
| Influenza A/H1N1                         | 61 (10/51)             | 238 (52/186)           | 0.70 (0.33–1.48)         | 0.64 (0.29–1.39)                 | 0.2588           |
| Influenza A/H3N2                         | 14 (2/12)              | 238 (52/186)           | 0.60 (0.13–2.75)         | 0.48 (0.10–2.28)                 | 0.3540           |
| Influenza B                              | 58 (6/52)              | 238 (52/186)           | 0.41 (0.17–1.01)         | 0.29 (0.11–0.78) *               | 0.0140 *         |
| Age 0–6 years \(^b\)                     |                        |                        |                          |                                  |                  |
|                          | Count | Total | **95% CI** | **95% CI** | P-value |
|--------------------------|-------|-------|------------|------------|---------|
| **Influenza A (total)**  |       |       |            |            |         |
|                          | 31 (5/26) | 137 (23/114) | 0.95 (0.33–2.74) | 1.00 (0.33–3.05) | 0.9990 |
| **Influenza A/H1N1**     |       |       |            |            |         |
|                          | 25 (5/20)  | 137 (23/114) | 1.24 (0.42–3.64) | 1.28 (0.41–3.98) | 0.6673 |
| **Influenza A/H3N2**     |       |       |            |            |         |
|                          | 6 (0/6)   | 137 (23/114) | Not available | Not available | Not available |
| **Influenza B**          |       |       |            |            |         |
|                          | 13 (0/13)  | 137 (23/114) | Not available | Not available | Not available |

**Age 7–15 years**

|                          | Count | Total | **95% CI** | **95% CI** | P-value |
|--------------------------|-------|-------|------------|------------|---------|
| **Influenza A (total)**  |       |       |            |            |         |
|                          | 44 (7/37) | 101 (29/72) | 0.47 (0.19–1.17) | 0.43 (0.16–1.14) | 0.0890 |
| **Influenza A/H1N1**     |       |       |            |            |         |
|                          | 36 (5/31)  | 101 (29/72) | 0.40 (0.14–1.13) | 0.39 (0.13–1.15) | 0.0878 |
| **Influenza A/H3N2**     |       |       |            |            |         |
|                          | 8 (2/6)    | 101 (29/72) | 0.83 (0.16–4.34) | 0.72 (0.13–3.95) | 0.7006 |
| **Influenza B**          |       |       |            |            |         |
|                          | 45 (6/39)  | 101 (29/72) | 0.38 (0.15–1.00) | 0.34 (0.12–0.94) | 0.0368 * |

CI: confidence interval

* statistically significant
a Influenza A (total) includes influenza A/H1N1 and A/H3N2.

b Fourteen patients were excluded (13: Not born before the previous season, 1: Unknown status of influenza infection during the previous season).

c One patient was excluded (1: Unknown status of influenza infection during the previous season).

d Odds ratios among all participants were adjusted for sex, age group (0–6 vs. 7–15 years), period of influenza infection onset (December 2019–January 2020 vs. February–March 2020), and influenza vaccination during the current season. Odds ratios in each age group were adjusted for sex, period of influenza infection onset (December 2019–January 2020 vs. February–March 2020), and influenza vaccination during the current season.

e P value is value of adjusted odds ratio.
Table 5 Odds ratio of influenza infection during the 2019–2020 season in relation to the presence or absence of influenza vaccination during the previous season

| Influenza vaccination during previous season | Test-positive (Yes/No) | Test-negative (Yes/No) | Crude Odds ratio (95% CI) | Adjusted Odds ratio (95% CI) | P-value |
|---------------------------------------------|------------------------|------------------------|--------------------------|-----------------------------|---------|
| Overall                                     |                        |                        |                          |                             |         |
| Influenza A (total)                         | 75 (33/42)             | 237 (134/103)          | 0.60 (0.36–1.02)         | 0.84 (0.40–1.76)             | 0.6406  |
| Influenza A/H1N1                            | 61 (25/36)             | 237 (134/103)          | 0.53 (0.30–0.95) *       | 0.75 (0.34–1.64)             | 0.4710  |
| Influenza A/H3N2                            | 14 (8/6)               | 237 (134/103)          | 1.02 (0.34–3.05)         | 1.35 (0.33–5.63)             | 0.6759  |
| Influenza B                                 | 58 (24/34)             | 237 (134/103)          | 0.54 (0.30–0.97) *       | 1.38 (0.53–3.61)             | 0.5058  |
| Age 0–6 years                               |                        |                        |                          |                             |         |
| Influenza Type       | Total Cases | Specimen Cases | Odds Ratio (95% CI) | p-value |
|---------------------|-------------|----------------|---------------------|---------|
| **Influenza A (total)** | 31 (14/17)  | 135 (83/52)    | 0.52 (0.23–1.13)    | 1.12 (0.33–3.79) | 0.8542 |
| Influenza A/H1N1    | 25 (12/13)  | 135 (83/52)    | 0.58 (0.25–1.36)    | 1.24 (0.34–4.51) | 0.7491 |
| Influenza A/H3N2    | 6 (2/4)     | 135 (83/52)    | 0.31 (0.06–1.77)    | 0.86 (0.06–13.55) | 0.9175 |
| Influenza B         | 13 (4/9)    | 135 (83/52)    | 0.28 (0.08–0.95) *  | 3.19 (0.38–27.04) | 0.2864 |

**Age 7–15 years**

| Influenza Type       | Total Cases | Specimen Cases | Odds Ratio (95% CI) | p-value |
|---------------------|-------------|----------------|---------------------|---------|
| **Influenza A (total)** | 44 (19/25)  | 102 (51/51)    | 0.76 (0.37–1.55)    | 0.81 (0.31–2.12) | 0.6702 |
| Influenza A/H1N1    | 36 (13/23)  | 102 (51/51)    | 0.57 (0.26–1.24)    | 0.62 (0.22–1.73) | 0.3661 |
| Influenza A/H3N2    | 8 (6/2)     | 102 (51/51)    | 3.00 (0.58–15.57)   | 2.97 (0.43–20.73) | 0.2718 |
| Influenza B         | 45 (20/25)  | 102 (51/51)    | 0.80 (0.40–1.62)    | 1.18 (0.40–3.45) | 0.7677 |

CI: confidence interval

* statistically significant
a Influenza A (total) includes influenza A/H1N1 and A/H3N2.

b Sixteen patients were excluded (13: Not born before the previous season, 3: Unknown status of influenza vaccination during the previous season).

c Odds ratios among all participants were adjusted for sex, age group (0–6 vs. 7–15 years), period of influenza infection onset (December 2019–January 2020 vs. February–March 2020), and influenza vaccination during the current season. Odds ratios in each age group were adjusted for sex, period of influenza infection onset (December 2019–January 2020 vs. February–March 2020), and influenza vaccination during the current season. In analysis of influenza H3N2 among patients aged 0–6 years, onset of influenza infection was excluded from the variables because there were no patients with influenza infection during the February–March 2020 period.

d $P$ value is value of adjusted odds ratio.