

# Influenza Virus Detection Following Administration of Live-Attenuated Intranasal Influenza Vaccine in Children With Cystic Fibrosis and Their Healthy Siblings

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**Background.** We aimed to explore the detection profile of influenza viruses following live-attenuated intranasal influenza vaccination (LAIV) in children aged 2–19 years with and without cystic fibrosis (CF).

**Methods.** Before the 2013–2014 influenza season, flocked nasal swabs were obtained before vaccination and 4 times in the week of follow-up from 76 participants ( $n_{CF}$ : 57;  $n_{healthy}$ : 19). Influenza was detected by reverse transcription polymerase chain reaction (RT-PCR) assays. A Bayesian hierarchical logistic regression model was used to estimate the effect of CF status and age on influenza detection.

**Results.** Overall, 69% of the study cohort shed influenza RNA during follow-up. The mean duration of RT-PCR detection was 2.09 days (95% credible interval [CrI]: 1.73–2.48). The odds of influenza RNA detection on day 1 following vaccination decreased with age in years (odds ratio [OR]: 0.82 per year; 95% CrI: 0.70–0.95), and subjects with CF had higher odds of influenza RNA detection on day 1 of follow-up (OR: 5.09; 95% CrI: 1.02–29.9).

**Conclusion.** Despite the small sample size, our results indicate that LAIV vaccine strains are detectable during the week after LAIV, mainly in younger individuals and vaccinees with CF. It remains unclear whether recommendations for avoiding contact with severely immunocompromised patients should differ for these groups.

**Keywords.** childhood vaccination; cystic fibrosis; influenza; live-attenuated influenza virus vaccine; viral detection.

Viral respiratory infections in pediatric patients with cystic fibrosis (CF) significantly exacerbate pulmonary problems and impact both short-term and long-term pulmonary function. This causes an increase in frequency and duration of hospitalizations [1–4], rendering vaccination against respiratory viruses—especially influenza, the most frequent respiratory virus that is vaccine-preventable—important in this population. Flumist, a live-attenuated influenza virus vaccine (LAIV) administered by intranasal spray, was approved for use in Canada

for individuals aged 2–59 years in June 2010. LAIV was introduced into publicly funded vaccination programs in Canada in the 2012–2013 influenza season. The intranasal route of administration of LAIV directly stimulates mucosal immunity in the nasopharynx of recipients, mimicking a naturally occurring influenza virus infection [5]. The ease of administration of LAIV can help increase uptake of seasonal influenza vaccination in pediatric populations [6]. However, a concern associated with the administration of LAIV is the shedding, and thus the potential transmission, of LAIV viruses from recent vaccinees to other individuals, in particular to severely immunocompromised patients. To date, there has been only one documented case of secondary transmission of LAIV identified among children attending daycare [7]. Yet, the Canadian National Advisory Committee on Immunization recommends that LAIV recipients avoid close contact with severely immunocompromised individuals for 2 weeks following vaccination [8].

Cystic fibrosis is characterized by, among other things, abnormal inflammatory signalling [9, 10], excessive neutrophil-dominated airway inflammation [11, 12], and impairment in the resolution of inflammation [13, 14]. These characteristics may impact the replication and detection of respiratory viruses like influenza from individuals with CF [15, 16]. We

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hypothesized that patients with CF may thus respond differently to influenza viruses, including live-attenuated strains, compared with individuals without CF. Whereas prolonged respiratory virus shedding after a naturally acquired infection in patients with asthma relative to healthy controls has been previously observed [17–21], no research exists evaluating viral detection after LAIV in patients with CF, which could be used as a proxy for naturally acquired infections. Also, limited research exists evaluating the relative duration of shedding following naturally acquired viruses in patients with CF [22]. Our objective was thus to explore the detection profile of influenza RNA in children and adolescents with CF following vaccination with LAIV. We sought specifically to understand the incidence and duration of influenza virus detection following vaccination and to explore the potential age dependency and the effect of CF status on the detection of influenza in recent LAIV vaccinees.

## METHODS

### Study Population

Eligible study participants aged 2–19 years were approached for study recruitment during the regular process of care for CF management approximately 2 months before the 2013–2014 influenza season. Two CF clinics in Canada participated in the study: The British Columbia Children’s Hospital (BC) and The Montreal Children’s Hospital (MCH). Healthy siblings (with no chronic respiratory or other conditions) of recruited patients with CF were also approached for enrollment. All potential participants had no contraindications to LAIV (Supplementary Appendix A). The study protocol for data collection was approved by the research ethics boards of both participating sites. The research ethics board of the McGill University Health Center approved the protocol for this analysis.

### Vaccination

LAIV was administered to participants as a 0.1-mL spray in each nostril after receiving informed consent. Each dose contained approximately  $10^{6.5-7.5}$  fluorescent focus units of each of the 3 recombinant strains for the 2013–2014 influenza season: A/California/7/2009 (H1N1)pdm09-like virus, A/Texas/50/2012-like virus, and B/Massachusetts/2/2012-like virus [23]. Participants were followed for 7 days from the time of vaccination because previous pediatric studies showed that the highest titers of influenza virus shed after LAIV were within this time period [24–26]. Flocked nasal swabs (Copan Diagnostics Inc, Murrieta, CA) were taken from participants immediately before vaccination on day 0 (D0) by a trained research nurse. Proper specimen collection technique was taught to participants (and/or their parents), and nasal samples were subsequently self-obtained on days 1, 2, 4, and 7 after vaccination (D1–D7, respectively). Swabs were immediately placed in 2.0 mL of universal transport medium (Copan) and then kept in home freezers and delivered frozen to the hospital sites. The specimens

were then stored at  $-80^{\circ}\text{C}$ , and all virologic testing was performed at the Laboratoire de Santé Publique du Québec.

### Laboratory Methods

Adhering to the protocol outlined in the World Health Organization’s guidelines for the molecular diagnosis of influenza virus in humans [27], reverse transcription polymerase chain reaction (RT-PCR) assays targeting influenza A and influenza B were used to identify and confirm the presence of influenza RNA in samples. Nucleic acid extraction was performed on the NucliSENS easyMag platform (bioMérieux, St. Laurent, Canada) according to the manufacturer’s instructions. The human enzyme ribonuclease P (RNP) gene was used as an internal positive control for the RT-PCR assays to determine the presence of human nucleic acids in the samples and to confirm successful nucleic acid extraction. Detection of influenza A and B viruses and of the RNP gene was performed on an iQ5 real-time PCR detection system (Bio-Rad, Mississauga, Canada) using the QuantiTect probe RT-PCR kit (QIAGEN, Toronto, Canada) (see “Description of Primer Sets Used in the Detection of Influenza Virus Genetic Material, RT-PCR Assay,” Supplementary Appendix B). The cycle threshold (Ct) of the RT-PCR reaction refers to the number of cycles of PCR required for the fluorescent signal to cross the background level and, although it is not a concentration itself, is roughly inversely proportional to the initial concentration of the target. Specimens with Ct values  $>37$  in the first RT-PCR run were confirmed in a second RT-PCR run. Specimens that tested positive in both runs were considered positive even if the Ct values obtained were  $>37$  (upper bound of 40). RT-PCR quantification standards were not run in parallel with the influenza A and B assays, precluding the quantification of viral load.

### Statistical Methods

Descriptive statistics and graphics were created using Stata 13. Proportions and means were estimated using binomial and normal models, respectively. Inferences including 95% credible intervals (CrIs) and risk differences (RDs) were calculated by the Gibbs sampler algorithm as implemented by WinBUGS (Version 1.4.3, MRC Biostatistics Unit, Cambridge, UK), which was also used to fit the hierarchical models described below. Markov Chain Monte Carlo (MCMC) convergence was assessed by visual inspection of history, trace, and quantile plots.

A hierarchical logistic regression model was used to estimate the odds ratios (ORs) of possible predictors of RT-PCR–detected influenza RNA (type A and/or B) for each subject and at each follow-up time. This model was used because it accounts for variation both within and between participants and controls for the correlation between observations, which arises when data are collected at several time points from the same individual. This model assumes a log-linear relationship between influenza detection and time and was chosen given that a maximum of only 4 time points of follow-up were available for each

participant (days 1, 2, 4, and 7). The first level of our model was a logistic regression with detection as the outcome for each time point of follow-up and for each subject, with subject-specific intercepts and slopes providing the probability of detection on the logit scale over time. At the second level of our model, the individual intercepts from the first level were modeled as a linear function of age and CF status (see “Additional Information Regarding Statistical Methodology Used,” [Supplementary Appendix C](#)). Univariable linear regression models first evaluated the individual effects of age (continuous), sex (dichotomous), CF status (dichotomous), and prior influenza vaccination (dichotomous) on the intercept for time ( $\alpha_i$ ) to determine which variables would be included in the final regression model. Inclusion in the final model was based on how reasonably the effect of each predictor variable on the outcome could be estimated based on CrI width (precision). Samples negative for influenza RNA were assigned a value of 0. Multiple imputation was used to account for missing outcome values; all covariate data were complete.

## RESULTS

### Demographic Characteristics

Seventy-six subjects (59 with CF) attending the 2 CF clinics ( $n_{BC} = 47$ ;  $n_{MCH} = 29$ ) were enrolled and vaccinated with LAIV between October 8 and November 28, 2013 (Table 1). The mean age of the cohort was 10.2 years, 51% of participants were female, and 95% of the cohort was vaccinated against influenza in the year before study participation. Of those immunized against influenza in 2012, 40% had been vaccinated with LAIV, and the remainder had received the trivalent inactivated influenza vaccine (TIV), administered by intramuscular injection. Five nasal swabs were anticipated from each participant, for a total of 380 samples. Overall, 372 samples were received:

**Table 1. Demographic Characteristics of Study Participants**

Characteristic	No. (%)		
	All	CF	Healthy
Health Status			
n/N (%)	76	59 (78%)	17 (22%)
Age, y			
Range	2–19	2–19	4–19
Mean (SD)	10.20 (4.98)	10.25 (5.07)	9.99 (4.78)
Median	10.00	10.80	9.10
Interquartile range	(5.65–14.20)	(5.40–14.50)	(6.60–12.50)
Sex			
Female	39 (51%)	28 (47%)	11 (65%)
Study site			
BC Children’s Hospital	47 (62%)	30 (51%)	17 (100%)
Montreal Children’s Hospital	29 (38%)	29 (49%)	0 (0%)
Influenza vaccine in the previous year (2012)			
LAIV	29 (38%)	29 (49%)	0 (0%)
TIV	43 (57%)	29 (49%)	14 (82%)
None	4 (5%)	1 (2%)	3 (18%)

Abbreviations: BC, British Columbia; CF, cystic fibrosis; LAIV, live-attenuated intranasal influenza vaccination; SD, standard deviation; TIV, trivalent inactivated influenza vaccination.

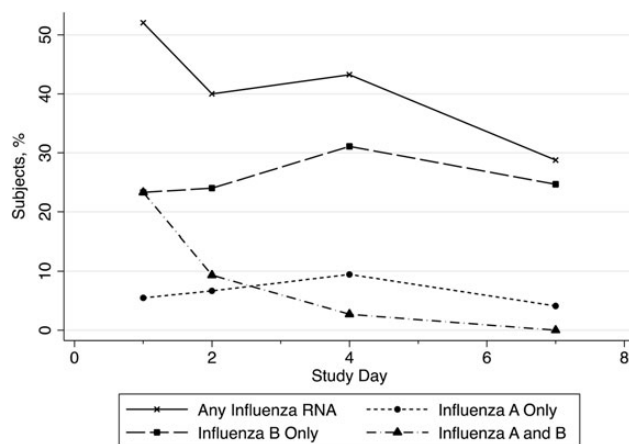
one participant was lost to follow-up after D0, and 4 participants were missing swabs for one day of follow-up.

No systematic difference in deterioration of samples was found between the MCH and BC study sites, as indicated by  $Ct_{RNP}$  values, thought to reflect sample quality (Figure 1B; [Supplementary Appendix C](#)). The  $Ct_{RNP}$  values appeared stable for each study day (Figure 1C; [Supplementary Appendix C](#)). Compliance with the study protocol for participant nasal swab collection was high: of the self-obtained samples, only 0.7% (2/304) did not have any detectable levels of influenza RNA or RNP (1 sample each on D1 and D7) and were thus considered missing. The majority of the samples contained a detectable amount of human DNA: only 1 of 372 samples had a very low amount of RNP ( $Ct_{RNP} > 37$ ).

### RT-PCR

Overall, influenza RNA was detected at least once during follow-up from 52 of 75 participants (69%; 95% CrI: 58%–79%). Specifically, in the entire study cohort, influenza A and B RNA were both detected at least once throughout the study period in 21 of 75 participants (28%; 95% CrI: 19%–39%), only influenza A was detected at least once in 7 of 75 (10%; 95% CrI: 4%–17%), only influenza B was detected at least once in 24 of 75 (32%; 95% CrI: 22%–43%), and no viral RNA was detected during follow-up in 23 of 75 (31%; 95% CrI: 21%–42%). Furthermore, influenza RNA was detected in 41 of 58 participants with CF (70%; 95% CrI: 58%–81%) and 11 of 17 healthy participants (64%; 95% CrI: 41%–84%) overall during the week following vaccination (RD: 6.38; 95% CrI: –16.92 to 31.49). Detection of only influenza B was most common in participants both with CF (28% influenza B vs. 9% only influenza A) and without CF (47% only influenza B vs. 12% only influenza A).

The mean total number of nasal swab specimens positive for any RT-PCR–detected influenza RNA (either type A and/or type B) per subject was 1.61 (95% CrI: 1.34–1.91). The mean



**Figure 1.** Number of subjects with RT-PCR–detected influenza RNA, by influenza virus type and study day.

**Table 2. Summary of Influenza Detection After Vaccination With Live-Attenuated Intranasal Influenza Vaccination in Study Cohort (Days 1–7 of Follow-Up)**

Influenza Virus	Total (n = 75) <sup>a</sup>	CF (n = 58)	No CF (n = 17)
Subjects from which any virus was detected, No. (%)	52 (69%)	41 (71%)	11 (65%)
Total detection episodes	58	46	12
<b>Duration</b>			
1 d	24 (41%) <sup>b</sup>	17 (37%)	7 (58%)
2 consecutive days	15 (26%)	11 (24%)	4 (33%)
3 consecutive days	9 (16%)	9 (20%)	0 (0%)
4 consecutive days	10 (17%)	9 (20%)	1 (8%)
Average duration of detection, mean (95% CrI)	2.09 (1.73–2.48)	2.22 (1.81–2.67)	1.58 (0.95–2.37)

Abbreviations: CF, cystic fibrosis; CrI, credible interval.

<sup>a</sup> One subject was lost to follow-up.

<sup>b</sup> Percentage of the total number of detection episodes that were of a particular duration; may not sum 100 due to rounding.

duration of RT-PCR–detected influenza RNA in the entire cohort was 2.09 days (95% CrI: 1.73–2.48). In participants with CF, the mean detection duration was 2.22 days (95% CrI: 1.81–2.67) compared with 1.58 days (95% CrI: 0.95–2.37) in healthy participants (Table 2), a difference of 0.63 (95% CrI: –0.25 to 1.41). The number of participants from whom influenza RNA was detected was highest on D1 for both influenza A and B viruses regardless of health status, and the proportion of participants from whom influenza was detected appeared to be decreasing on the last day of follow-up, D7 (Figure 1).

### Predictors of Influenza Detection

The effects of age and CF status on the detection of influenza RNA on D1 could be estimated from the univariable hierarchical logistic regression models (Table 3A). Based on the final, hierarchical logistic multivariable regression model (Table 3B), the odds of detecting influenza RNA on D1 after LAIV decreased with increasing age in years (OR: 0.82; 95% CrI: 0.70–0.95). Furthermore, subjects with CF had higher odds of having influenza RNA detected on D1 after LAIV compared with healthy participants (OR: 5.09; 95% CrI: 1.02–29.9). The odds of detecting influenza RNA decreased with each day of follow-up (OR: 0.67; 95% CrI: 0.50–0.87).

We observed that the number of participants from whom influenza RNA was detected was highest on D1 for both influenza A and B viruses, which could represent either remnant from the LAIV administered on the previous day or actively replicating virus. We conducted two post hoc sensitivity analyses to better understand D1 influenza detection. First, the hierarchical logistic regression model described above was run excluding D1 data for all participants (Table 3C). Subsequently, a simple logistic regression model was run evaluating the effect of age and CF status on the detection of any influenza RNA during D2–D7 of follow-up (Table 3D). CF status and younger age were consistently observed to increase the odds of detection of influenza virus after LAIV in both of these supplementary analyses, indicating that the increase observed for D1 in the final multivariable model (Table 3B) was likely replicating virus.

### DISCUSSION

Influenza RNA was detected at least once in the week following vaccination from the nasal swabs of almost 70% of the study

**Table 3. Hierarchical Logistic Regression Models for the Outcome of Detection of Influenza A or B Virus**

(3a) Univariable <sup>a</sup> Models for the Intercept, $\alpha_i$ (Day 1)			(3b) Multivariable Model for the Intercept, $\alpha_i$ (Day 1)		(3c) Sensitivity Analysis: Multivariable Model for the Intercept, $\alpha_i$ (Day 2):		(3d) Sensitivity Analysis: Simple Logistic Regression Model (Days 2, 4, and/or 7 of Follow-up)	
Variable		OR (95% CrI)	Variable	OR (95% CrI)	Variable	OR (95% CrI)	Variable	OR (95% CrI)
Health Status	CF	4.64 (0.90–26.79)	CF	5.09 (1.02–29.93)	CF	5.34 (1.08–33.95)	CF	1.75 (0.49–6.28)
	Healthy	Referent	Healthy	Referent	Healthy	Referent	Healthy	Referent
Age <sup>b</sup>		0.83 (0.72–0.96)	Age <sup>b</sup>	0.82 (0.70–0.95)	Age <sup>b</sup>	0.80 (0.6–0.92)	Age <sup>b</sup>	0.79 (0.70–0.88)
Sex	Female	0.44 (0.10–1.82)	Intercept ( $\alpha_i$ )	2.71 (0.39–19.30)	Intercept ( $\alpha_i$ )	2.87 (0.41–22.18)	Intercept ( $\alpha$ )	12.35 (2.58–66.67)
	Male	Referent	Time ( $\beta_i$ )	0.69 (0.50–0.87)	Time ( $\beta_i$ )	0.73 (0.53–0.94)		
LAIV vaccine in 2012 <sup>c</sup>	Yes	2.34 (0.53–10.94)						
	No	Referent						
TIV vaccine in 2012 <sup>c</sup>	Yes	0.57 (0.13–2.43)						
	No	Referent						
Age (quadratic polynomial)								
Age		1.84 (0.97–3.62)						
Age <sup>2</sup>		0.96 (0.93–0.99)						

Abbreviations: CF, cystic fibrosis; CrI, credible interval; OR, odds ratio; LAIV, live-attenuated intranasal influenza vaccination; TIV, trivalent inactivated influenza vaccination.

<sup>a</sup> Each variable was included independently at the second level of the hierarchy, as predictors of each subject  $i$ 's intercept ( $\alpha_i$ ).

<sup>b</sup> Modeled continuously (linear).

<sup>c</sup> The referent category for each of these 2 independent, dichotomous variables is not receiving the vaccine in 2012 (ie, previous influenza season).

cohort. In comparable pediatric studies, the proportion of LAIV vaccinees shedding influenza ranged between 7% and 80% in cohorts of children with and without chronic conditions [24–26, 28–30]. We also found that patients with CF had higher odds of influenza detection on the first and second day following immunization compared with their healthy counterparts. In previous studies evaluating LAIV in children, peak shedding of LAIV-strain viruses occurred on D2 following vaccination or later [24, 25, 28–30]; these studies did not, however, include patients with CF. Previously evaluated pathophysiologic mechanisms potentially explaining differences in viral detection between patients with and without CF include less interferon-related antiviral gene induction and greater inflammatory cytokine gene induction in patients with CF [31], elevated interleukin-8 and dampened apoptotic responses in airway epithelial cells of patients with CF [32], and absence of nitric oxide synthesis in individuals with CF [11], all of which relate to an individual's ability to respond to viral infections.

The inversely proportional relationship between influenza RNA detection and age that was observed in this study cohort, albeit with a wide but statistically significant CrI, is consistent with findings from previous similar studies [24, 26, 33]. Factors that may affect the incidence and duration of viral detection after immunization with LAIV relate to an individual's immunity to the virus, which is largely influenced by prior influenza vaccinations and naturally acquired influenza infections (the likelihood of which increases with age). The decrease in detection of influenza virus with increasing age may be a marker of the decreased efficacy of LAIV seen in older individuals [34–37]. Notably, influenza type B was more commonly detected than type A, regardless of CF status. Similar findings were reported by Vesikari et al [25] in a pediatric trial that compared LAIV vs placebo. Increased mucosal immunity against influenza A in may also help explain the discrepancy between influenza types shed by participants in this cohort [33].

Results from this study must be interpreted in light of several limitations. First, the sample size precluded a definitive conclusion of whether CF status affects the duration of detection of influenza RNA in recent LAIV vaccinees. Rather these results allow an exploratory analysis of this association. Second, the primers used in the RT-PCR were not specific to LAIV strains. It is therefore possible that the influenza viruses detected from the nasal swab samples were naturally circulating viruses rather than LAIV. However, specimen collection from study subjects occurred well before the widespread circulation of wild-type influenza viruses in 2013 (Supplementary Appendix D). Third, 6 participants ( $n_{BC} = 3$  and  $n_{MCH} = 3$ ) had nonconsecutive detection of the same influenza type during follow-up (all 6 participants had positive D1 and D4 samples but negative D2 samples). The intermittent detection is likely due to a viral concentration close to (or below) the RT-PCR assay's level of

detection. Fourth, a recent study in ferrets determined that antigen detection and virus culture, but not RT-PCR, could identify the end of the infectious period of influenza [38]. As such, PCR assays cannot discriminate between infective virions and noninfective viral nucleic acids, precluding the extrapolation of our results to contagiousness of recent LAIV vaccinees. Fifth, the overall sensitivity of influenza virus detection has been shown to be higher from nasopharyngeal aspirate compared with nasal swab specimens [39, 40]. The use of nasopharyngeal swabs may thus have increased the sensitivity of detection and better reflected the replication of influenza virus compared with remnant RNA in the nasal cavity, the site of administration of LAIV. Finally, previous studies have established a potential association between host genetics and susceptibility to influenza infection [41, 42]. If there exists a similar genetic component to the types of detection outcomes evaluated in this study, the decreased genetic variability in our study cohort (arising from the recruitment of siblings of participants with CF) would limit the generalizability of study findings to individuals with a similar genetic makeup.

Overall, we found that detection of influenza viruses in pediatric patients with and without CF occurred up to at least 7 days following administration of LAIV. The mean duration of detection of influenza RNA was roughly 2 days following immunization with LAIV, regardless of health status, and the odds of detection decreased with each day following immunization. Although the RT-PCR detection of influenza RNA does not necessarily equate to shedding due to active replication of the virus and perhaps equates even less to contagiousness of the individual and risk of transmission [25], our results indicate that LAIV vaccine strains are detectable during the week following administration. We also determined decreasing odds of influenza detection with increasing age, in years. As hypothesized, patients with CF had higher odds of influenza detection on both the first and second days following LAIV administration compared with healthy participants. Further research is thus required to conclusively determine whether younger LAIV vaccinees and individuals with CF require a different recommendation for contact with severely immunocompromised patients. Future projects may build on this research to explore the effects of seropositivity and serosusceptibility to influenza (which vary with age) on LAIV-strain virus detection. Further research is also necessary to consolidate the specific pathophysiologic mechanisms behind seemingly differential responses to virus detection in patients with CF. Results of this study also help to better understand the epidemiology of the vaccine virus and provide fundamental information for mathematical modeling exercises of LAIV-strain influenza transmission.

### Supplementary Data

Supplementary material is available online at *Open Forum Infectious Diseases* online (<http://OpenForumInfectiousDiseases.oxfordjournals.org/>).

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