Randomized evaluation of live attenuated vs. inactivated influenza vaccines in schools (RELATIVES) cluster randomized trial: Pilot results from a household surveillance study to assess direct and indirect protection from influenza vaccination

Jeffrey C. Kwong, Jennifer A. Pereira, Susan Quach, Rosana Pellizzari, Edwina Dusome, Margaret L. Russell, Jemila S. Hamid, Yael Feinberg, Anne-Luise Winter, Jonathan B. Gubay, Brittany Sirtonski, Deanna Moher, Doug Sider, Michael Finkelstein, Mark Loeb, for the Public Health Agency of Canada/Canadian Institutes of Health Research Influenza Research Network (PCIRN) Program Delivery and Evaluation Group

**A R T I C L E   I N F O**

Article history:
Received 8 April 2015
Received in revised form 14 July 2015
Accepted 16 July 2015
Available online 29 July 2015

Keywords:
Influenza
Influenza vaccines
Live vaccines
Canada
Ontario

**A B S T R A C T**

*Background:* Children are key drivers of influenza transmission. Vaccinating school age children decreases influenza in the community.

*Objective:* To pilot-test the methods for a future trial to compare the direct and indirect benefits of inactivated influenza vaccine (IIV) vs. live attenuated influenza vaccine (LAIV) in preventing influenza infection.

*Methods:* During the 2013–14 influenza vaccination campaign, we piloted an open-label cluster randomized trial involving 10 elementary schools in Peterborough, Ontario, Canada. We randomized schools on a 1:1 basis to have students receive IIV or LAIV. We invited a subset of vaccinated students and their households to participate in a surveillance sub-study, which involved completing daily symptom diaries during influenza season and collecting mid-turbinate swabs from symptomatic individuals to detect influenza infection. The main outcome measure was confirmed influenza infection using a real-time reverse transcriptase polymerase chain reaction (PCR) assay.

*Results:* One hundred and nineteen households (166 students and 293 household members) participated. During 15 weeks of surveillance, we detected 22 episodes of PCR-confirmed influenza (21 influenza A/H1N1 and 1 influenza B). The incidence of influenza per 1000
1. Introduction

School children drive influenza epidemics through virus transmission to their contacts [1–4]. Mathematical models and field research suggest that vaccinating school children provides indirect protection (herd immunity) to both household members and the community at large [5–9], thereby reducing the burden of influenza. However, influenza vaccine coverage in children is sub-optimal for various reasons, including accessibility, competing demands, and fear of needles among children [10–12].

An alternative to injectable inactivated influenza vaccine (IIV) is the intranasal, live attenuated influenza vaccine (LAIV). LAIV was first approved for use in the United States in 2003 for individuals aged 5–49 years, and extended to those aged 2–49 years in 2007 [13]. In Canada, LAIV was approved for use in June 2010, and for the 2011–12 to 2013–14 influenza seasons Canada’s National Advisory Committee on Immunization preferentially recommended it over IIV for healthy children aged 2–17 years based on efficacy, effectiveness, and immunogenicity [14–17]. However, the extent of indirect protection from LAIV for reducing the incidence of laboratory-confirmed influenza among household contacts has not yet been established.

In the fall of 2013, we piloted a cluster randomized trial to evaluate administering LAIV vs. IIV to children at school-based influenza immunization clinics. A cluster design was used because the intervention was at the level of the school. This paper describes a nested sub-study involving students who were vaccinated as part of the larger study. We conducted surveillance of the vaccinated students and their households for influenza infection in order to assess feasibility of study procedures and generate parameter estimates to inform a full-scale trial evaluating the direct and indirect benefits of LAIV.

2. Methods

We conducted an open-label cluster randomized trial involving 10 elementary schools within the geographic boundaries of the Peterborough County–City Public Health Unit (PCCHU) during the 2013–14 influenza vaccination campaign. PCCHU is the local public health department that serves a mixed urban-rural community 125 km northeast of Toronto, Ontario. Out of the 28 schools belonging to the Kawartha Pine Ridge District School Board, 10 agreed to participate. Using a standard computer pseudorandom number generator, researcher JAP randomized the schools on a 1:1 basis to having students between Junior Kindergarten (age 4) and Grade 8 (age 13) offered free LAIV (FluMist®) or IIV (Vaxigrip®) at PCCHU-organized school-based immunization clinics between 11 and 22 November 2013. Both vaccines contained A/California/7/2009 (H1N1) X-179A, A/Texas/50/2012 (H3N2) X-223A, and B/Massachusetts/2/2012 (Yamagata lineage) BX-518 viruses. Details are described elsewhere [18].

We invited 320 households (with 429 school-vaccinated students from 9 of the 10 schools to participate in this study). One IIV-assigned school, representing 11 households with 20 vaccinated students, was excluded from the study due to distance from the city of Peterborough and the related challenges of reaching them for follow-up in the winter. The study involved monitoring for acute respiratory symptoms among all household members and collecting specimens from symptomatic participants to test for influenza during the period of local influenza activity.

2.1. Recruitment

Between 6 December 2013 and 1 February 2014, research assistants attempted to contact each vaccinated student’s parent(s) by telephone to participate. Repeated calls were made to those who could not be reached, until the end of the recruitment period. We limited recruitment from each school to a maximum of 25 households. A research assistant visited interested households to obtain written consent from each adult (aged ≥ 16 years) in the family and assent from each child younger than 16 years. Households were offered an incentive of a $25 Amazon.ca gift card per participant.

2.2. Study procedures

Once consent/assent was obtained for the household, a research assistant recorded baseline data from each household member, including demographics, risk factors for influenza complications, and current influenza vaccination status. We provided each household with either paper or electronic diaries (via a link to an Internet-based questionnaire) for recording daily symptoms, a digital thermometer, Copan flocked nasal swabs, and training on the collection of mid-turbinate swabs from oneself or other household members.

We instructed participating households to complete the daily diary to record whether any household member had acute respiratory symptoms, illness history (e.g., hospitalizations related to lower respiratory tract infections and pneumonia, physician visits for respiratory illness), and missed days of school or work due to acute respiratory infections. If any household member exhibited any one of cough, sore throat, or fever, or at least two other symptoms (runny nose, headache, sinus problems, muscle aches, fatigue/very tired, ear ache, ear infection, chills, and sneezing), they were to collect a mid-turbinate swab on themselves (or parents would swab children) as soon as symptoms appeared (within 48 h), and to call a research assistant to collect a second mid-turbinate swab. Thus there were typically two swabs per episode of illness, so that we could compare participant collection with research assistant collection. A participant was considered to have had a positive test if either of the two specimens was positive for influenza. A repeat swab was collected 7 days after the collection of the initial swab (indicating a new episode) if the individual had at least person-days was 1.24 (95% CI, 0.40–2.89) for IIV-vaccinated students, compared to 0.13 (95% CI, 0.003–0.72) for LAIV-vaccinated students; the incidence rate ratio was 0.10 (95% CI, 0.002–0.94). Similarly, the incidence of influenza per 1000 person-days was 1.33 (95% CI, 0.64–2.44) for IIV household members, compared to 0.47 (95% CI, 0.17–1.03) for LAIV household members; the incidence rate ratio was 0.36 (95% CI, 0.11–1.08). The overall incidence rate ratio (combining students and household members) was 0.27 (95% CI, 0.09–0.69).

Conclusions: Household surveillance involving participant monitoring and reporting of symptoms and self-collection of mid-turbinate swabs is feasible. A larger study is required to validate the suggestion that vaccinating children with LAIV might confer more protection against influenza for both children and their household contacts, compared to IIV.

Trial registration: ClinicalTrials.gov NCT01995851. © 2015 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
one additional symptom, as well as the initial two symptoms; or if the individual had at least two new symptoms (different from the initial two symptoms and the first two had resolved).

Data collection began on 16 December 2013, when the weekly percentage of clinical and public health laboratory tests positive for influenza from PCCHU and the three adjacent health departments (Durham Region Health Department, Hastings and Prince Edward Counties Health Unit, and Haliburton, Kawartha, Pine Ridge District Health Unit) exceeded 5%. The surveillance period continued for 15 weeks, ending on 24 March 2014.

During the surveillance period, research assistants contacted each household that opted for paper diaries twice weekly by telephone to obtain data for each household member, and recorded this information directly into the survey software program FluidSurveys (Ottawa, Canada). Households that opted for electronic diaries received a daily link to the patient diary. We emailed the main contact of each household, who completed the form on behalf of all household members. If no entries were submitted for a household for three consecutive days, we called to remind them to complete the diary.

The research assistants collected all nasal swabs during household visits and brought them to the regional laboratory in Peterborough, where they were stored in a refrigerator until they were shipped by courier at refrigeration temperature to the central Public Health Ontario laboratory in Toronto for testing. The specimens were tested by real-time reverse transcription polymerase chain reaction (rtRT-PCR) using protocols from the U.S. Centers for Disease Control and Prevention (CDC) to detect the presence of influenza A (M gene) or B (NS1 gene) virus. Subtyping for seasonal influenza A/H3 was performed by real-time RT-PCR targeting the HA gene (CDC protocol) and A(H1N1)pdm09 was confirmed using an in-house real-time RT-PCR assay [19].

2.4. Ethics

This study was approved by Public Health Ontario’s Ethics Research Board and the Kawartha Pine Ridge District School Board’s Research Advisory Committee.

3. Results

One hundred and twenty-two households agreed to participate. Three households withdrew from the study within one week of enrolment. In total, we analyzed data from 119 households (166 students and 293 household members), including 42 households (57 students and 108 household members) with a student who received IV at school (“IV households”), and 77 households (109 students and 185 household members) with a student who received LAIV at school (“LAIV households”) (Fig. 1). The mean number of household members was 3.9 for IV households and 3.8 for LAIV households.

Students from both groups were similar in age, sex, body mass index, and risk factors for influenza complications. However, a lower percentage of IV-vaccinated students had received influenza vaccination the previous season (2012–13) compared to LAIV-vaccinated students (32.4 vs. 51.6%; p = 0.02) (Table 1). When we examined children younger than nine years of age, IV-vaccinated students were less likely to have been vaccinated against influenza during the 2012–13 season compared to LAIV-vaccinated students (25.9 vs. 41.3%). However, parents of IV-vaccinated students were

![Fig. 1. Diagram showing the flow of schools through the cluster randomized trial.](image-url)
more likely than parents of LAIV-vaccinated students to report their child’s vaccination status for the 2012–13 season as “unknown” (38.7 vs. 12.7%).

Household members from both groups were similar except for a trend toward fewer members of IVV households having been vaccinated against influenza during the 2013–14 season compared to members of LAIV households (35.2 vs. 44.9%; \( p = 0.10 \)).

We detected 22 episodes of PCR-confirmed influenza during the study period (21 influenza A/H1N1 and 1 influenza B). One household had 3 cases, 4 households had 2 cases, and 11 households had a single case. We observed 15 episodes of PCR-confirmed influenza in IVV households compared to 7 episodes in LAIV households (Table 2). The incidence of influenza per 1000 person-days was 1.24 (95% CI, 0.40–2.89) for IVV-vaccinated students, compared to 0.13 (95% CI, 0.003–0.72) for LAIV-vaccinated students; the incidence rate ratio was 0.10 (95% CI, 0.002–0.94). Similarly, the incidence of influenza per 1000 person-days was 1.33 (95% CI, 0.64–2.44) for IVV household members, compared to 0.47 (95% CI, 0.17–1.03) for LAIV household members; the incidence rate ratio was 0.36 (95% CI, 0.11–1.08). The overall incidence rate ratio (combining students and household members) was 0.27 (95% CI, 0.09–0.69). The in-school correlation within schools was 0.07.

IVV households had significantly fewer unobserved days at the start of the surveillance period compared to LAIV households (41.7 vs. 46.3 days, respectively; \( p = 0.001 \)). We observed no differences between the IVV and LAIV groups in terms of the proportion of symptomatic participants who were swabbed (68.2 vs. 73.9%; \( p = 0.31 \)), or the proportion of swabbed individuals who fulfilled the case definition (98.4 vs. 99.6%; \( p = 0.38 \)). The mean duration between participant collection and research assistant swab collection was 1.6 days (range 0–7 days; standard deviation 1.34 days), with 83% of research assistant swabs collected within 2 days of participant swabs. Of the 22 episodes of documented influenza infection, no swab was collected by a research assistant for 4 episodes. Of the remaining 18 episodes, we observed concordance between the 2 swabs for 12 episodes (with a mean delay between participant collection and research assistant collection of 0.75 days, range 0–2 days), and discordance for 6 episodes (with mean delay of 2 days, range 0–4 days). Among the discordant episodes, 4 episodes were detected only by the participant and 2 episodes were detected only by the research assistant. If the study had involved only swab collection by participants, we would have detected 90.9% of the total influenza episodes (20/22).

The influenza test-positive and test-negative participants were similar, except that the former group was significantly less likely to have been vaccinated against influenza (with either vaccine) during the 2013–14 season (4.5 vs. 27.4%; \( p = 0.02 \)) or the 2012–13 season (4.5 vs. 40.2%; \( p < 0.001 \) (Table 3).

### 4. Discussion

This pilot study was conducted to assess the feasibility of study procedures and to generate parameter estimates to inform a future full-scale trial comparing the direct and indirect benefits of immunizing children against influenza with LAIV compared with IVV. We demonstrated that a surveillance study involving vaccinated students and their household members monitoring for symptoms, reporting using daily diaries, and self-collecting mid-turbinate...
Table 2
Incidence rates and incidence rate ratios of laboratory-confirmed influenza.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Students</th>
<th>Household members</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IV n = 57</td>
<td>LAIV= n = 109</td>
<td>IV n = 108</td>
</tr>
<tr>
<td>Number of influenza infections</td>
<td>5 (8.8)</td>
<td>1 (0.9)</td>
<td>10 (9.3)</td>
</tr>
<tr>
<td>Uninfected person-days of follow-up</td>
<td>4044</td>
<td>7715</td>
<td>7541</td>
</tr>
<tr>
<td>Incidence of influenza per 1000 person-days (95% CI)</td>
<td>1.24 (0.40–2.89)</td>
<td>0.13 (0.003–0.72)</td>
<td>1.33 (0.64–2.44)</td>
</tr>
<tr>
<td>Incidence rate ratio (LAIV/IV) (95% CI)</td>
<td>0.10 (0.002–0.94)</td>
<td>0.36 (0.11–1.08)</td>
<td>0.27 (0.09–0.69)</td>
</tr>
</tbody>
</table>

* Inactivated influenza vaccine; † Live attenuated influenza vaccine; and ‡ Number of laboratory-confirmed influenza infections/1000 uninfected person-days.

Table 3
Characteristics of those who tested positive for influenza compared to those who tested negative for influenza.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Influenza positives n = 22 (%)</th>
<th>Influenza negatives n = 437 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Students</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 3 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4–13 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14–17 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 18–19 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than high school</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school graduation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-secondary education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight (&lt;18.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (18.5–24.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight (25.0–29.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obese (≥30.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk factors for influenza complications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic diseasesb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aboriginal status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthcare worker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Has a regular doctor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinated against influenza, 2012–13 season</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinated against influenza since October 2013</td>
<td>1 (4.5)</td>
<td>120 (27.4)</td>
</tr>
</tbody>
</table>

* Categories for children are based on the 2000 Centers for Disease Control and Prevention Growth Reference in the United States; † Chronic diseases included: asthma, diabetes, cancer, anemia, heart disease, lung disease, kidney disease, and blood diseases; some categories were excluded if they were 0% (e.g., aspirin user).

Swabs within 48 h is feasible. Interestingly, our results provide suggestive evidence of direct benefits that are greater than have been previously estimated, as well as possible indirect protection from vaccinating school children with LAIV rather than IV. The direct benefits of LAIV vs. IV in children are well established. A meta-analysis of 3 clinical trials involving 13,000 healthy children aged 6 months to 17 years demonstrated that compared to IV, receipt of LAIV reduced the risk of laboratory-confirmed influenza by 45–53% in vaccine-naive children (aged 6–71 months) and 35% in previously vaccinated older children (aged 6–17 years) [17].

Our study suggested a 90% reduction in influenza incidence among LAIV-vaccinated children compared with IV-vaccinated children, but the confidence interval was very wide due to the small sample size.

The indirect benefits of IV have been demonstrated most definitively to date in a cluster randomized trial of rural Hutterite religious communities [6]. Vaccinating 83% of school age children reduced the risk of influenza infection among other community members (who were generally not vaccinated) by 61%. An earlier RCT demonstrated that household contacts of IV-vaccinated pre-school aged children were less likely to experience febrile respiratory illnesses [20]. Further, observational studies that have examined the impact of immunizing children have found that this resulted in significant reductions in episodes of influenza-like illnesses and influenza-related complications in the elderly and adult populations [21–23].

Whether LAIV confers greater indirect benefits than IV remains uncertain. Piedra et al. conducted a nonrandomized community-based influenza vaccine trial of both vaccines during the 2003–04 season and found that only certain age groups (adults aged 35–44 years and children aged 5–11 years) experienced lower rates of medically-attended acute respiratory illness during the epidemic in the intervention vs. control community, based on a coverage of 32% [23]. They did not assess the indirect benefits in a head-to-head comparison. The results of our study suggest that household members of LAIV-vaccinated children were 64% less likely to experience influenza infection than household members of IV-vaccinated children, although once again the confidence interval was very wide.

Strengths of this study include the rigorous follow-up with daily symptom monitoring, the use of PCR-confirmed influenza as the outcome, and the use of local viral surveillance data to determine the surveillance period.

This study had a number of limitations. First, this was an open-label trial, which may have introduced bias because both participants and research staff were aware of the type of vaccine received by the subjects. However, the proportion of symptomatic participants who were swabbed and the proportion of swabbed individuals who fulfilled the case definition were comparable across both groups (although a slightly higher proportion of symptomatic participants in the LAIV group was swabbed), suggesting that knowledge of vaccine type did not substantially influence the likelihood of specimen collection. Second, the relatively low participation rate of the households may have introduced selection bias and impacted generalizability of the findings, but the participation rate was similar across the two arms. Third, due to the brief interval between the school immunization clinics and the start of influenza season, household recruitment continued for the first 7 weeks of the surveillance period, and we noted a greater degree of delay in recruiting LAIV households than IV households. As most of the IV households enrolled prior to the peak of influenza activity whereas the majority of the LAIV households enrolled following the peak, and consequently a greater proportion of swabbing episodes for the IV group occurred earlier than the LAIV group, we may have incompletely captured influenza infections among the LAIV households, thereby leading to biased estimates of vaccine effectiveness. Fourth, incomplete ascertainment of influenza infections may have occurred due to participant fatigue, refusal to be swabbed (particularly in young children), or suboptimal identification of influenza infection among infants using the provided symptom list (e.g., irritability was not included on the list). However, each of these issues should have affected both arms equally. Fifth, we stopped the study early and missed an influenza B outbreak that occurred over the course of 10 weeks (24 March 2014–31 May 2014) following the
end of this study, Sixth, this study was intended to be a pilot, so the small sample size precluded adjustment for any potential confounders. Seventh, changes in household members’ influenza vaccination status may not have been reported, which could have introduced misclassification bias. Finally, since we observed a trend toward higher IV coverage among LAIV household members, it may not be possible to determine whether the reduced incidence of influenza in LAIV household members is attributable to indirect protection from vaccinating students with LAIV or direct protection from receipt of IV among household members.

A future study would be strengthened by incorporating the following features: (1) incorporating blinding of both participants and investigators by using suitable placebos (i.e., saline nasal sprays for those assigned to IV, and saline intramuscular injections for those assigned to LAIV); (2) scheduling vaccination clinics as early as possible to minimize vaccinations occurring in non-school settings; (3) optimizing strategies to recruit households into the study; and (4) using other data sources to ascertain influenza vaccination status over the course of the influenza season to mitigate incomplete reporting by participants.

5. Conclusion

The results of our pilot study demonstrate that a household surveillance study involving participant monitoring and reporting of symptoms and self-collection of mid-turbinate swabs is feasible. A larger study is required to validate the suggestion that vaccinating children with LAIV might confer more protection against influenza for both children and their household contacts, compared to IV.

Funding

Public Health Agency of Canada/Canadian Institutes of Health Research Influenza Research Network and Public Health, Ontario, Canada.

Conflicts of Interest

J. Gubbay has received research grants from GlaxoSmithKline Inc. and Hoffman-La Roche Ltd. (>3 years ago) to study antiviral resistance in influenza, and from Pfizer Inc. to conduct microbiological surveillance of Streptococcus pneumoniae.

The full trial protocol may be requested from the authors.

Acknowledgments

We wish to thank the Ontario Ministry of Health and Long-Term Care, the Peterborough County-City Health Unit, the Kawartha Pine Ridge District School Board, the staff at the Public Health Ontario laboratories in Peterborough and Toronto, the principals and teachers at the participating schools, and especially the students and their parents for their participation in this study. This study was supported by an operating grant from the Public Health Agency of Canada and the Canadian Institutes of Health Research (CIHR) (grant number PIR 124309), and in-kind funding from Public Health Ontario. Dr. Kwong was supported by a CIHR New Investigator Award and a University of Toronto Department of Family and Community Medicine Clinician Scientist Award. The Canadian Association for Immunization Research and Evaluation provided networking assistance.

References