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Effect of inactivated influenza vaccination on human coronavirus infection: Secondary analysis of a randomized trial in Hutterite colonies

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ABSTRACT

Background: Although influenza vaccines provide protection against influenza viruses, concern has been raised that they may increase susceptibility to non-influenza respiratory viruses. As pandemic lockdowns end, temporal overlap of circulation of seasonal influenza viruses and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is expected. Understanding the impact of influenza vaccination on risk of coronavirus infection is therefore of considerable public health importance.

Methods: We performed a secondary analysis of a randomized trial where children and adolescents in Canadian Hutterite colonies were randomly assigned by colony to receive the 2008–2009 seasonal inactivated trivalent influenza vaccine (TIV) or a control hepatitis A (HepA) vaccine. All 3273 colony members (vaccinated children and nonvaccine recipients) were followed for the primary outcome of RT-PCR confirmed seasonal coronavirus infection. Serum collected pre- and post-vaccination was analyzed for titers of IgG antibodies towards human coronaviruses (HCoV).

Results: The incidence of coronavirus infection was 0/18/1000 person-days in the colonies that received TIV vs 0.36/1000 person-days in the control group, hazard ratio (HR) 0.49 [0.21–1.17]. The risk reduction among non-vaccine recipients in the TIV group compared to the control group was HR 0.55 [0.24–1.23]. There was an increase in the geometric mean fold change of HCoV-OC43 antibody titers following TIV compared to HepA vaccine (mean difference 1.2 [0.38–2.06], p = 0.007), and an increase in geometric mean HCoV-NL63 antibody titers post-TIV (262.9 vs 342.9, p = 0.03).

Conclusion: The influenza vaccine does not increase the risk of a coronavirus infection. Instead, the influenza vaccine may reduce the rate of coronavirus infections by inducing cross-reactive anti-coronavirus IgG antibodies.

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1. Background

Influenza is a major cause of mortality, resulting in an estimated 300,000–600,000 annual deaths worldwide [1]. As a result, countries worldwide, including U.S.A and Canada, recommend the influenza vaccine for all individuals 6 months and older without any contraindications [2–4]. The effect of the influenza vaccine on susceptibility to coronaviruses is, however, unclear. Some observational studies have shown that children receiving the influenza vaccine are at an increased risk of an acute respiratory illness from non-influenza respiratory viruses [5–7]. Vaccine-associated virus interference has been proposed as a mechanism to explain this observation, wherein vaccinated individuals may be at an increased risk of respiratory virus infection because they do not receive the non-specific immunity associated with the immune response to natural infection. In contrast, other studies have shown that the influenza vaccine may have a protective effect against coronavirus infections [8]. It has been proposed that this could...
be due to a phenomenon termed bystander activation, the activation of antibody-producing cell that recognize non-vaccine antigens [9].

Given that SARS-CoV-2 is expected to continue to circulate after the pandemic during the influenza season, understanding the effect of influenza vaccination on human coronavirus infection is of considerable public health importance. This also has implications for susceptibility of vaccinated individuals during outbreaks of SARS-CoV, Middle East respiratory syndrome coronavirus (MERS-CoV), and the four species of human coronaviruses (HCoV-OC43, HCoV-NL63, HCoV-HKU1, and HCoV-229E) that circulate seasonally.

The majority of Hutterite colonies are in the Canadian provinces of Alberta, Saskatchewan, and Manitoba. Although single families live on the colonies in their own homes, many activities are communal, including dining. These colonies are isolated from major cities and towns but there is sufficient interaction such that respiratory viruses are regularly introduced into the colonies.

We conducted a secondary analysis using data from a randomized trial conducted during the 2008–2009 influenza season in Hutterite colonies where children and adolescents were randomly assigned by colony to receive the seasonal inactivated trivalent influenza vaccine (TIV) or a control hepatitis A (HepA) vaccine [10]. We hypothesized that receiving the influenza vaccine would not increase the risk of coronavirus infections and may reduce infection.

2. Methods

Our primary objective was to determine the incidence of laboratory-confirmed seasonal coronavirus infections in the TIV group compared to the control HepA vaccine group. The secondary objectives included measuring serum anti-coronavirus binding antibodies, evaluating the indirect effectiveness of the TIV on coronavirus infection risk, and determining the incidence of other non-influenza respiratory viruses.

2.1. Study design and participants

Participants were 3273 residents of 46 Hutterite colonies from Alberta, Saskatchewan, and Manitoba, Canada enrolled in a cluster randomized trial from September 22 to December 23, 2008, as previously reported [10]. Children and adolescents in Hutterite colonies were randomized by colony to receive TIV or HepA vaccine as a control. Pre- and post-vaccination serum was collected from children and adolescents who directly received the TIV or HepA vaccine. Other colony members were enrolled to assess the indirect effectiveness of the influenza vaccine. Children and adolescents who were allocated to the influenza vaccine group received the formulation recommended for the 2008–2009 influenza season (A/Brisbane/59/2007 [H1N1]-like virus, A/Brisbane/10/2007 [H3N2]-like virus, B/Florida/4/2006-like virus; Vaxigrip, Sanofi Pasteur, Lyon, France). Children and adolescents who were allocated to the control vaccine were immunized with the hepatitis A vaccine (Aavaxim-Pediatric, Sanofi Pasteur). The original study received ethics approval [10]. For this report, we used sera already collected and re-analyzed it to broaden our understanding of influenza vaccination and its relationship to seasonal coronaviruses. Since this contributes to the original research question, we did not require specific ethics approval for this.

2.2. Laboratory confirmation of respiratory viruses

Participants were assessed for signs and symptoms of respiratory illness over the follow-up period, defined by the surveillance period of December 28, 2008 until June 23, 2009 [10]. Nasopharyngeal specimens were obtained if there was documented influenza-like illness (ILI), defined as the presence of two or more of the following symptoms: fever (≥38 °C), cough, nasal congestion, sore throat, headache, sinus problems, muscle aches, fatigue, earache or infection, or chills [10]. Influenza and non-influenza respiratory viruses were detected using the Centers for Disease Control and Prevention Human Influenza Virus Real-time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) Detection and Characterization Panel in 2009 [10]. The panel includes HCoV-229E, HCoV-NL63, HCoV-OC43, parainfluenza virus type 1, 2, 3, 4, enterovirus, respiratory syncytial virus (RSV) A and B, and human metapneumovirus.

2.3. Recombinant antigen production

Plasmid encoding mammalian cell codon optimized sequences for the receptor binding domain (RBD) of SARS-CoV-2 was generously gifted from the lab of Dr. Florian Krammer (Icahn School of Medicine, NYC) [11]. The sequence for the HCoV-OC43 RBD was obtained from the UniProt Protein Database (P36334 SPIKE_CV-HOC). This construct was engineered to contain an N-terminal 13 amino acid signal sequence and a C-terminal 6xHis-tag for downstream protein purification. Mammalian cell codon optimization was performed using the GenScript GenSmart Codon Optimization Tool. The RBD gene was synthesized with GenScript and cloned into the pcDNA3.1 plasmid between EcoRI and XhoI restriction enzyme sites. The constructs for HCoV-229E and HCoV-NL63 RBD were designed similarly using UniProt Protein Database. Proteins were produced in Exp293 cells (ThermoFisher) using the manufacturers’ instructions. Proteins were purified, concentrated, and analyzed by SDS-PAGE.

2.4. Enzyme Linked Immunosorbent Assay (ELISAs)

To assess whether TIV non-specifically induced antibodies against human coronaviruses, we interrogated serum IgG levels in paired pre- and post-vaccination samples (N = 14). All samples were stored at −80 °C with limited freeze thaw cycles. Due to limited sample availability, only 13 samples from the HepA vaccine group, and 12 from the TIV group were assessed against SARS-CoV-2 RBD. ELISAs were performed as previously described [12]. In brief, plates were coated overnight with 2 μg/mL of recombinant RBD from HCoV-OC43, HCoV-229E, HCoV-NL63, or SARS-CoV-2 viruses. After blocking with 5% skim milk in PBS-T (0.1% Tween-20), participants’ pre- and post-vaccination serum was serially diluted in skim milk from a starting dilution of 1:10 and added to the plate to incubate for 1 h at room temperature. A goat anti-human IgG-horseradish peroxidase (HRP) conjugated secondary antibody (Santa Cruz Biotechnology, Dallas, TX, USA) in conjunction with horseradish peroxidase substrate (Sigmafast OPD, Sigma Aldrich, St. Louis, MO, USA) was used to develop the reaction. Optical density (O.D.) at 490 nm was read on a Spectramax i3 (Molecular Devices, San Jose, CA, USA). As a control for non-specific binding of secondary antibody, one column of wells received only secondary antibody to serve as a plate blank. Serum IgG endpoint titers were defined by the lowest dilution at which the O.D. was three standard deviations above the mean of the blank wells.

2.5. Statistical analysis

Incidence was calculated as person-days. Only the first episode detected was used in the analysis to avoid lack of independence associated with counting multiple outcomes. To determine the effect of influenza vaccination on coronavirus infection, we used a Cox proportional hazards regression model, using robust
sandwich variance estimates to account for the effect of clustering. We adjusted the hazard ratio with the inclusion of a covariate for external influenza immunization of nonrecipients. For the ELISAs, participants’ pre- and post-vaccination sera were randomly selected for analysis of antibody titers (N = 14). Analysis of pre- and post-vaccination reciprocal endpoint titers was conducted by paired parametric t-tests. The geometric mean log2 fold change ratios of anti-coronavirus IgG titers pre- to post-vaccination were analyzed as a mean difference (MD) between the TIV and HepA vaccine group. All p values and 95% confidence intervals (CIs) were calculated with 2-tailed tests. Differences with p < 0.05 were considered significant. All statistical analyses were conducted using the open software R version 4.0.4 (R Foundation for Statistical Computing, Vienna, Austria) or GraphPad Prism version 9.1.1.

3. Results
3.1. Study participants

There were 3273 participants, 1773 of whom resided in colonies randomized to receive the seasonal TIV and 1500 of whom resided in colonies randomized to receive the HepA vaccine as a control (Fig. 1). Characteristics of the colonies were similar in the two

![Flow diagram of trial and secondary analysis of coronavirus infections.](image-url)
groups (Table 1). Approximately 58.9% of participants were 15 years of age or younger in the influenza vaccine group and 57.3% in the control group. Co-morbidities were similar between both groups. There were 123 individuals amongst both groups (3.8% of 3273) with at least 1 episode of laboratory-confirmed coronavirus infection (Table 1).

### 3.2. Detection of coronavirus and other non-influenza respiratory viruses

The incidence of seasonal coronavirus infections was higher in the HepA vaccine group (0.36/1000 person-days [0.28–0.44]) than the TIV group (0.18/1000 person-days [0.13–0.24]). The risk reduction of coronavirus infections in the TIV group was 51% (hazard ratio (HR) 0.49 [0.21–1.18]). There was a similar trend in all age groups, with individuals aged 30–39 having a significant 80% reduction in risk of coronavirus infections (p = 0.02) (Table 2).

When coronavirus subtypes were independently analyzed (Table 1), there was a lower incidence of each seasonal coronavirus virus detected in the HepA vaccine group compared to TIV (0 vs 13).

### Table 1

| Characteristics of participants. | Influenza Vaccine | Hepatitis A Vaccine |
|----------------------------------|-------------------|-------------------|
| Mean age (SD)                    | 25.9 (19.9)       | 25.9 (20.0)       |
| Age ≥ 16 (%)                     | 1045 (58.9)       | 860 (57.3)        |
| Female (%)                       | 1004 (56.6)       | 846 (56.4)        |
| Number of coronavirus positive swabs (%) | 46 (2.6)  | 77 (5.1) |
| Number of influenza positive swabs (%) | 80 (4.5)  | 159 (10.6) |
| Influenza-like illness (%)        | 60 (3.4)          | 87 (5.8)          |
| ≥ 1 Comorbidities (%)            | 199 (11.2)        | 173 (11.5)        |

≥ 1 Comorbidities refers to the presence of one or more of the following: Heart or lung disorders, blood disorders, swallowing or choking problems, chronic metabolic diseases, kidney or liver disease, and cancer, immunodeficiency, or immunosuppression.

### Table 2

| Age Group | Incidence* (95% CI) | N (%) | Hemagglutinin avidity |
|-----------|---------------------|-------|-----------------------|
| Total     | 0.18 (0.13–0.24)    | 46 (2.6) | 0.36 (0.28–0.44)     |
| 0 to 4    | 0.21 (0.08–0.47)    | 6 (0.4)  | 0.32 (0.14–0.63)     |
| 5 to 8    | 0.14 (0.04–0.35)    | 4 (0.2)  | 0.21 (0.07–0.50)     |
| 9 to 14   | 0.22 (0.10–0.41)    | 9 (0.5)  | 0.42 (0.25–0.68)     |
| 15 to 19  | 0.20 (0.06–0.46)    | 5 (0.3)  | 0.25 (0.08–0.59)     |
| 20 to 29  | 0.12 (0.03–0.30)    | 4 (0.2)  | 0.28 (0.11–0.58)     |
| 30 to 39  | 0.10 (0.02–0.28)    | 3 (0.2)  | 0.50 (0.26–0.88)     |
| 40 to 49  | 0.24 (0.10–0.49)    | 7 (0.4)  | 0.41 (0.21–0.71)     |
| >50       | 0.22 (0.10–0.44)    | 8 (0.5)  | 0.36 (0.18–0.64)     |

* Incidence is calculated as number of cases per 1000 person-days.

### Table 3

| Virus | Incidence (95% CI) | N (%) | Probability |
|-------|--------------------|-------|-------------|
| Influenza Vaccine | Hepatitis A Vaccine |
| 229E  | 0.08 (0.05–0.12)   | 20 (1.1) | 0.13 (0.09–0.19) | 30 (1.7) | 0.56 (0.21–1.51) | 0.25 |
| OC43  | 0.02 (0.004–0.04)  | 4 (0.2)  | 0.07 (0.04–0.11) | 15 (0.8) | 0.22 (0.04–1.19) | 0.08 |
| NL63  | 0.09 (0.06–0.13)   | 23 (1.3) | 0.15 (0.10–0.20) | 32 (1.8) | 0.60 (0.11–3.31) | 0.56 |
significant geometric mean difference (MD) in log2 fold change ratio of HCoV-OC43 RBD IgG titers when comparing serum from children and adolescents who received TIV to those who received HepA vaccine (1.07 vs $0.14$, MD $1.2$ $[0.38–2.06]$, $p = 0.007$) (Fig. 3A). There was a trend towards a significant difference in the geometric mean log2 fold change ratio against HCoV-NL63 RBD ($0.50$ vs $0.07$, MD $0.57$ $[0.05–1.19]$, $p = 0.07$) and HCoV-229E ($1.07$ vs $0.14$, MD $1.2$ $[-0.22–2.64]$, $p = 0.09$) (Fig. 3B,C). The geometric mean log2 fold change ratio against SARS-CoV-2 RBD ($0.83$ vs $0.66$, MD $0.17$ $[-1.30–1.63]$, $p = 0.82$) was not significantly different (Fig. 3D).

4. Discussion

Here, we show evidence that TIV provides a moderate reduction in the rate of seasonal coronavirus infections. In colonies randomized to receive TIV, the risk reduction in coronavirus infections was 51%. In these colonies, both children and adolescents who directly received the influenza vaccine and non-vaccinated members had a lower incidence of coronavirus detection by RT-PCR. Community vaccination with seasonal influenza vaccine has previously been shown by our group to provide indirect protection against influenza viruses to non-vaccinated community members [10]. The present data suggests that community influenza vaccination may be able to promote indirect protection against human coronaviruses as well. In our panel of viruses, the protection provided by TIV appeared to be specific to coronaviruses as a risk reduction was not observed in other non-influenza respiratory viruses. Taken together, this suggests that the risk reduction of coronavirus infection is less likely to be attributed to stochasticity. We further demonstrated that the protective effects observed following TIV vaccination was associated with non-specific activation of anti-coronavirus antibodies. This is consistent with other studies that have shown significant bystander B-cell activation following influenza vaccination [9]. In children and adolescents who received TIV, there was an increase in antibody titers against HCoV-NL63 post-vaccination and a greater mean fold change of HCoV-OC43 antibody levels when compared to those who received the HepA vaccine.

A growing body of evidence has shown that the influenza vaccine can reduce the rate of coronavirus infection and the severity of disease [13–15]. Indeed, recent studies have shown that the influenza vaccine is associated with reduced SARS-CoV-2 infections amongst healthcare workers [14,16]. In addition, ecological studies using public health data have demonstrated that SARS-CoV-2 infections are enriched in areas with less influenza vaccine uptake [17–20]. These corroborate with our findings that the influenza vaccine is protective against coronavirus infections. In contrast, a study among defense personnel showed that recipients of the influenza vaccine were at an increased risk of seasonal human coronaviruses [7]. Our findings, however, suggest that immunity derived from indirect activation of B-cells may be greater than the negative effects associated with vaccine-induced virus interfer-
ence. Unfortunately, current available evidence lacks a clear biological mechanism to explain the effects of influenza vaccine on coronavirus infection. Few studies have investigated the association between seasonal influenza vaccination and anti-coronavirus immune responses, and of those that have, results are conflicting and inconclusive [16,21,22].

Our findings suggest that the influenza vaccine induces activation of anti-coronavirus IgG antibodies. Vaccines are designed to induce long-lasting memory B-cells and T-cells such that a more rapid and robust immune response can be mounted against subsequent exposures to a specific pathogen. In addition to direct MHC-T-cell receptor (TCR) activation of antigen-specific T-cells, these cells can also be activated in a TCR-independent and cytokine-dependent manner. Activation through this pathway results in T-cells that lack specificity for a particular pathogen, yet can impact the pathogenicity through the induction of cross-reactive immunity [23]. Termed “bystander activation”, this effect has been demonstrated in the setting of humoral immunity, as polyclonal activation of human memory B-cells bearing non-specific responses to viral pathogens following vaccination has been documented [24]. Horns et al. showed that less than 60% of antibodies produced by memory B cells elicited by the 2011–2012 influenza vaccine exhibited binding to the vaccine strain, suggesting that significant bystander activation of B-cells can occur after influenza vaccination [9]. Thus, our findings suggest that the influenza vaccine may induce non-specific activation of B-cells and elicit antibodies that can bind to the RBD of seasonal coronaviruses, including HCoV-OC43 and HCoV-NL63. Although beyond the scope of this study, it is possible that these anti-coronavirus antibodies may also be cross-reactive to the RBD of influenza viruses, notably hemagglutinin. We did not observe a significant change in HCoV-229E and SARS-CoV-2 RBD IgG antibodies, which is likely due to low pre-vaccination antibody titers. Therefore, further randomized controlled trials (RCTs) investigating anti-coronavirus antibody repertoires after influenza vaccination will help to clarify whether these results can be interpreted in the context of the current pandemic.

There are two primary strengths to the present study. First, to the best of our knowledge, this is the first RCT to examine the effect of the influenza vaccine on the incidence of coronavirus infection. In contrast to the present study, ecological and observational studies are confounded by health behaviours that help to mitigate the spread of infections. Behaviours such as social distancing and masking may be more accepted amongst those who opt to receive the influenza vaccine. Our study is a RCT that is not confounded by these variables as TIV was compared to a control vaccine (HepA), all members were blinded, and social distancing and masking were not implemented in these colonies. Second, we used a serological assay to show quantitatively that the influenza vaccine induces the activation of anti-coronavirus IgG antibodies, providing a biologically-plausible mechanism that directly supports our clinical findings.

There are some limitations to the present study. First, RT-PCR for laboratory-confirmed respiratory viruses was only conducted when individuals had documented symptoms of an ILI. Seasonal coronaviruses have been detected in asymptomatic individuals,

Fig. 3. Log2 fold change ratios of anti-coronavirus IgG antibodies against receptor binding domains (RBDs) of (A) HCoV-OC43, (B) HCoV-NL63, (C) HCoV-229E, and (D) SARS-CoV-2 from pre-vaccination (baseline) to post-vaccination (follow-up) in the inactivated trivalent influenza vaccine (TIV) group and the hepatitis A (HepA) vaccine group.
thus asymptomatic carriers could have gone undetected [25]. However, given that asymptomatic children and adolescents are active transmitters of human coronaviruses, asymptomatic carriers are more likely to have been positive in the HepA vaccine group as these colonies had greater transmission rates and a higher incidence of symptomatic cases [26,27]. Second, the power of the primary study was calculated to assess whether vaccinating children or adolescents with the TIV could prevent influenza infection in other colony members [10]. Third, a low sample size was used for serological assessment of anti-coronavirus IgG antibodies. Therefore, this follow-up study may be limited by the sample size required to determine the effect of the influenza vaccine on coronavirus infections. Our data suggest that the effects of influenza vaccination should be tested in adequately powered studies in specific age groups. Nevertheless, we were able to observe a moderate reduction in coronavirus infections after influenza vaccination and a significant increase in antibodies against human coronaviruses.

5. Conclusion

The influenza vaccine does not increase the risk of seasonal coronavirus infections. Rather, seasonal influenza vaccines may be important in reducing the incidence of human coronavirus infections by inducing anti-coronavirus immunity. As pandemic lockdowns end, governments and healthcare providers should promote influenza vaccination to reduce the burden that will come with co-circulation of influenza virus and human coronaviruses. This would be predicted to enhance the effectiveness of current COVID-19 vaccines. Further RCTs will help reinforce the potential protective effects of the influenza vaccine on coronavirus infections.

Author contributions

ML and PS initiated, recruited, and coordinated the RCT. ML, MSM, HDS, AM, and ATC planned the study and analysis. PS and JA contributed to data management. ATC, PS, and HDS were responsible for drafting the manuscript. All authors revised the manuscript critically.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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