Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Vaccine 39 (2021) 7058–7065

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

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

Andrew T. Chen [a], Hannah D. Stacey [b,c,d], Art Marzok [b,c,d], Pardeep Singh [e], Jann Ang [b,c,d], Matthew S. Miller [b,c,d], Mark Loeb [a,c,e,f,g,*]

[a] Department of Medicine, McMaster University, Hamilton, ON, Canada
[b] Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON, Canada
[c] Michael G. DeGroote Institute for Infectious Disease Research, McMaster University, Hamilton, ON, Canada
[d] McMaster Immunology Research Centre, McMaster University, Hamilton, ON, Canada
[e] Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada
[f] Department of Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, ON, Canada
[g] Department of Mathematics and Statistics, McMaster University, Hamilton, ON, Canada

ARTICLE INFO

Article history:
Received 13 August 2021
Received in revised form 28 September 2021
Accepted 11 October 2021
Available online 18 October 2021

Keywords:
Influenza
Coronavirus
Vaccine
Clinical epidemiology
Bystander activation

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.

© 2021 Elsevier Ltd. All rights reserved.

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

* Corresponding author at: Department of Medicine, McMaster University, 1280 Main Street West, Hamilton, ON L8S 4Z1, Canada.
E-mail address: loebm@mcmaster.ca (M. Loeb).

https://doi.org/10.1016/j.vaccine.2021.10.021
0264-410X/© 2021 Elsevier Ltd. All rights reserved.

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 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 (Avaxim-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 ($\geq$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, entero/rhinovirus, 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_CVHOC). 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 by 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 Expi293 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

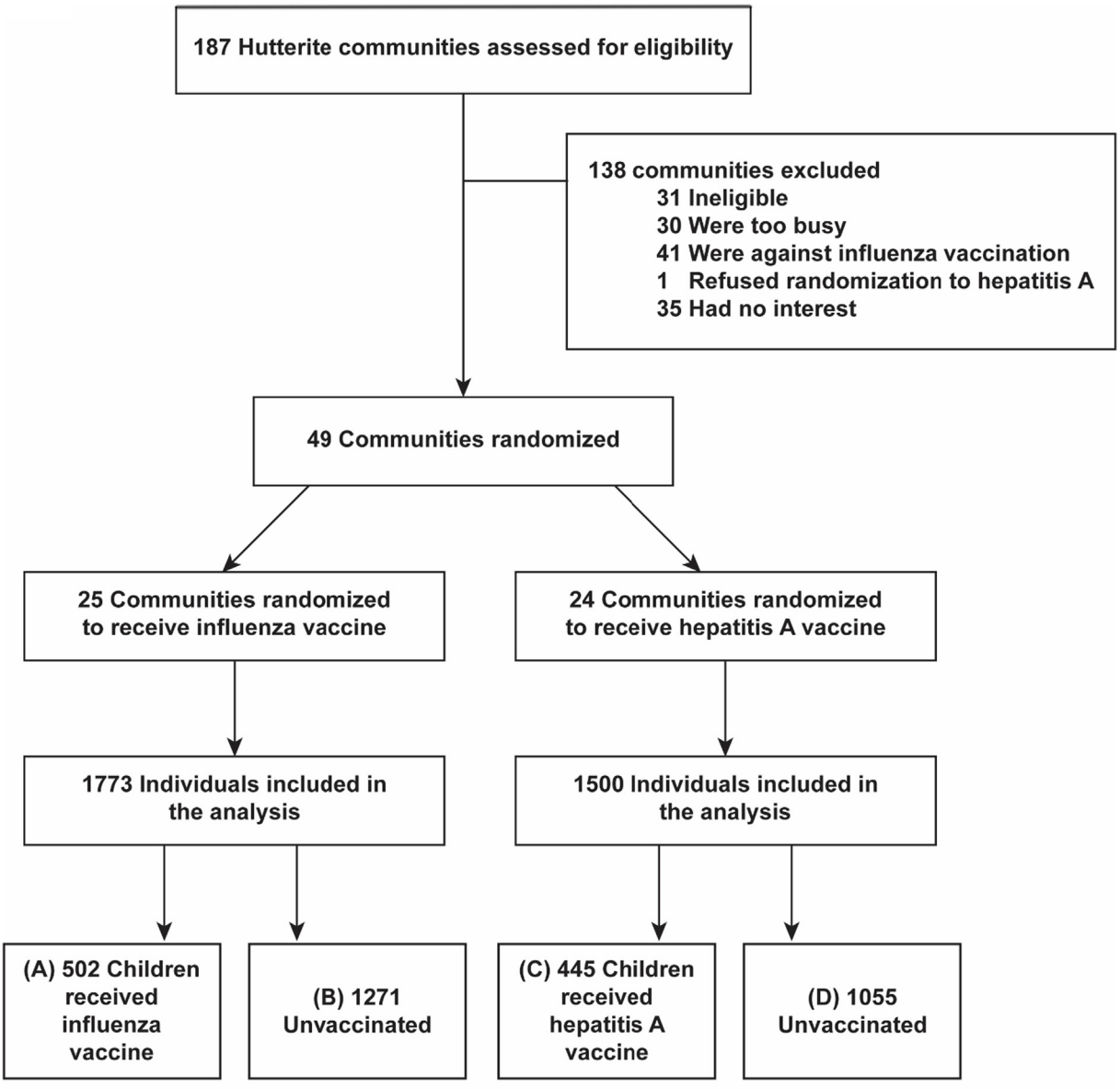
Incidence was calculated as person-1000 days. Only the first episode detected was used in the analysis to avoid lack of independence associated with counting multiple outcomes. To determine the effect of influenza vaccination on coronavirus infection, we used a Cox proportional hazards regression model, 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



Comparison	Influenza Vaccine	Control
All individuals	A+B	C+D
Direct vaccination	A	C
Indirect vaccination	B	D

Fig. 1. Flow diagram of trial and secondary analysis of coronavirus infections.

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 coronaviruses 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 3), there was a lower incidence of each seasonal coronavirus in the TIV group, with the greatest reduction seen in HCoV-OC43 cases (HR 0.22 [0.04–1.19]), followed by HCoV-229E (HR 0.56 [0.21–1.51], and HCoV-NL63 (HR 0.60 [0.11–3.31]).

The risk reduction of coronavirus infections was 61% amongst children and adolescents who directly received the TIV (HR 0.39 [0.12–1.24]). To determine the effect of the influenza vaccine on coronavirus rates amongst nonrecipients, we compared the incidence of those who did not receive the vaccine, but who resided in colonies randomized to intervention or control. The risk reduction of coronavirus infection in participants randomized to the intervention arm, but who did not directly receive the TIV was 45% (HR 0.55 [0.24–1.23].

In analyzing the incidence of other non-influenza respiratory viruses, we found no difference in the incidence of enterovirus and rhinovirus (HR 0.80 [0.45–1.41]), parainfluenza virus (HR 0.61 [0.20–1.85]), or RSV infections (HR 1.29 [0.48–3.49]) (supplementary Table 1). There were no cases of human metapneumovirus detected in the HepA vaccine group compared to TIV (0 vs 13).

3.3. Serological detection of anti-coronavirus antibodies

There was a higher geometric mean reciprocal endpoint IgG titer against HCoV-OC43 RBD following TIV, but this did not reach statistical significance (131.4 vs 301.4, p = 0.09) (Fig. 2A). In comparison, there was no increase in HCoV-OC43 RBD IgG in HepA vaccine recipients (120.7 vs 127.1, p = 0.48). There was a significant increase (262.9 vs 342.9, p = 0.03) in IgG titers against HCoV-NL63 RBD following TIV (Fig. 2B). There was no increase in IgG titers pre- to post-vaccination in the HepA vaccine group (368.5 vs 408.6, p = 0.64). We did not observe significant differences in the pre- and post-vaccination geometric mean reciprocal endpoint IgG titers against HCoV-229E RBD for HepA vaccine or TIV, p = 0.47 and p = 0.18, respectively (Fig. 2C). Similarly, no significant differences in geometric mean reciprocal endpoints were seen against SARS-CoV-2 RBD for either group (HepA vaccine, p = 0.16; TIV, p = 0.08) (Fig. 2D).

To more directly compare the induction of cross-reactive antibodies elicited by HepA vaccine or TIV, the log2 ratio of fold change following vaccination was examined. We observed a statistically

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 of 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
Incidence and hazard ratio of coronavirus infection in colonies randomized to receive the influenza vaccine or the hepatitis A vaccine.

	Influenza Vaccine		Hepatitis A Vaccine			
Age Group	Incidence[a] (95% CI)	N (%)	Incidence (95% CI)	N (%)	Hazard Ratio[b]	P value
Total	0.18 (0.13–0.24)	46 (2.6)	0.36 (0.28–0.44)	77 (5.1)	0.49 (0.21–1.17)	0.11
0 to 4	0.21 (0.08–0.47)	6 (0.4)	0.32 (0.14–0.63)	8 (0.5)	0.67 (0.21–2.17)	0.5
5 to 8	0.14 (0.04–0.35)	4 (0.2)	0.21 (0.07–0.50)	5 (0.3)	0.64 (0.10–3.96)	0.63
9 to 14	0.22 (0.10–0.41)	9 (0.5)	0.42 (0.25–0.68)	17 (1.1)	0.52 (0.18–1.54)	0.24
15 to 19	0.20 (0.06–0.46)	5 (0.3)	0.25 (0.08–0.59)	5 (0.3)	0.76 (0.2–2.86)	0.69
20 to 29	0.12 (0.03–0.30)	4 (0.2)	0.28 (0.11–0.58)	7 (0.5)	0.40 (0.09–1.74)	0.22
30 to 39	0.10 (0.02–0.28)	3 (0.2)	0.50 (0.26–0.88)	12 (0.8)	0.19 (0.05–0.77)	0.02
40 to 49	0.24 (0.10–0.49)	7 (0.4)	0.41 (0.21–0.71)	12 (0.8)	0.58 (0.23–1.43)	0.24
>50	0.22 (0.10–0.44)	8 (0.5)	0.36 (0.18–0.64)	11 (0.7)	0.56 (0.25–1.29)	0.18

[a] Incidence is calculated as number of cases per 1000 person-days.
[b] A robust sandwich variance estimator was used with Cox proportional hazards to adjust for membership in the randomized colonies.

Table 3
Incidence and hazard ratio of coronavirus subtype infection in colonies randomized to receive the influenza vaccine or the hepatitis A vaccine.

	Influenza Vaccine		Hepatitis A Vaccine			
Virus	Incidence (95% CI)	N (%)	Incidence (95% CI)	N (%)	Hazard Ratio	P value
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

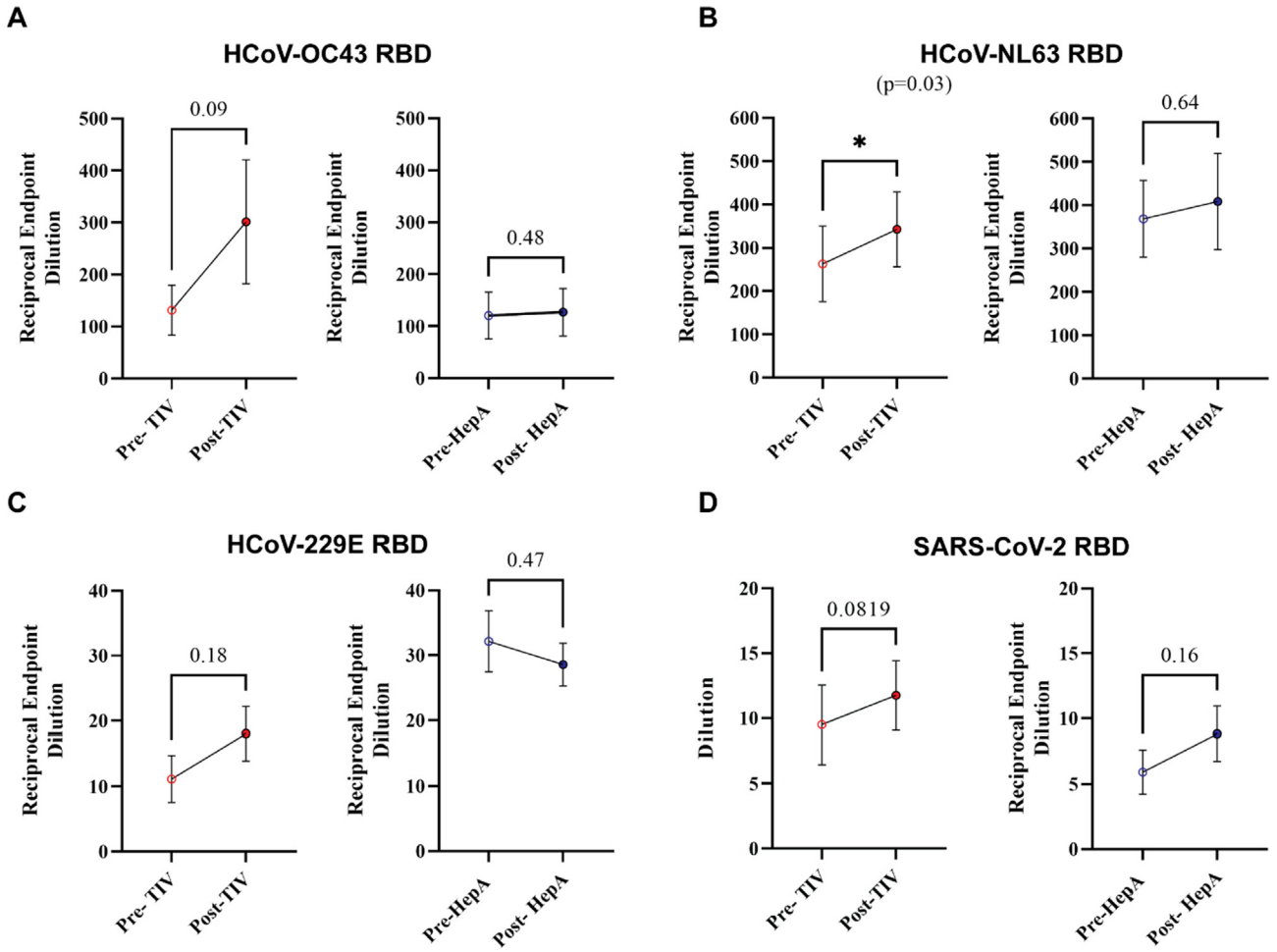


Fig. 2. Mean reciprocal endpoint IgG titers against the receptor binding domains (RBDs) of (A) HCoV-OC43, (B) HCoV-NL63, (C) HCoV-229E, and (D) SARS-CoV-2 pre- and post-vaccination of inactivated trivalent influenza vaccine (TIV) or hepatitis A (HepA) vaccine.

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-

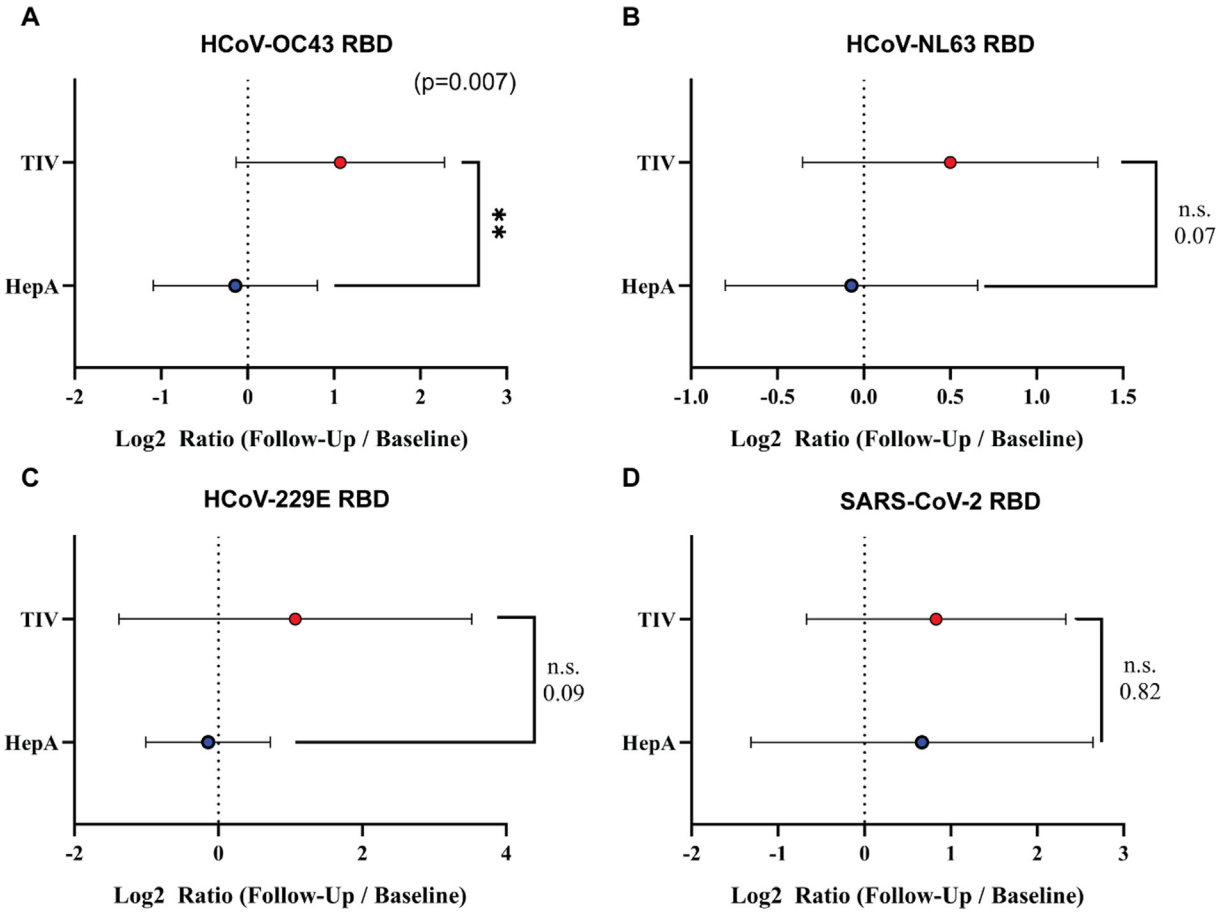


Fig. 3. Log2 fold change ratios of anti-coronavirus IgG antibodies against receptor binding domains (RBDs) of (A) HCoV-OC43, (B) HCoV-NL63, (C) HCoV-229E, and (D) SARS-CoV-2 from pre-vaccination (baseline) to post-vaccination (follow-up) in the inactivated trivalent influenza vaccine (TIV) group and the hepatitis A (HepA) vaccine group.

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,

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 statistical analysis. ATC, HDS, AM, MSM, and ML were responsible for drafting the manuscript. ML and PS supervised the community sites and contributed to data collection. 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.

Acknowledgements

The original trial was supported by CIHR. The secondary analysis did not receive dedicated funding. MSM was supported, in part, by a CIHR New Investigator Award and an Ontario Early Researcher Award. HDS was supported, in part, by an Ontario Graduate Scholarship and a Canadian Society for Virology United Supermarket Studentship. The corresponding author had full access to all the data in the study and had full responsibility for the decision to submit for publication. 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.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2021.10.021.

References

[1] Iuliano AD, Roguski KM, Chang HH, Muscatello DJ, Palekar R, Tempia S, et al. Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. Lancet 2018;391(10127):1285–300. https://doi.org/10.1016/S0140-6736(17)33293-2.
[2] Grohskopf LA, Sokolow LZ, Broder KR, Walter EB, Fry AM, Jernigan DB. Prevention and control of seasonal influenza with vaccines: Recommendations of the Advisory Committee on Immunization Practices-United States, 2018–19 influenza season. MMWR Recomm Reports 2018;67:1. , https://doi.org/10.15585/mmwr.rr6703a1.
[3] Langley JM, Faughnan ME. Prevention of influenza in the general population. CMAJ 2004;171:1213–22. https://doi.org/10.1503/cmaj.1031737.
[4] Young K, Gemmill I, Harrison R. Summary of the NACI seasonal influenza vaccine statement for 2020–2021. Canada Commun Dis Rep 2020;46:132–7. , https://doi.org/10.14745/ccdr.v46i05a06.
[5] Cowling BJ, Fang VJ, Nishiura H, Chan K-H, Ng S, Ip DKM, et al. Increased risk of noninfluenza respiratory virus infections associated with receipt of inactivated influenza vaccine. Clin Infect Dis 2012;54(12):1778–83. https://doi.org/10.1093/cid/cis307.
[6] Rikin S, Jia H, Vargas CY, Castellanos de Belliard Y, Reed C, LaRussa P, et al. Assessment of temporally-related acute respiratory illness following influenza vaccination. Vaccine 2018;36(15):1958–64. https://doi.org/10.1016/j.vaccine.2018.02.105.
[7] Wolff GG. Influenza vaccination and respiratory virus interference among Department of Defense personnel during the 2017–2018 influenza season. Vaccine 2020;38(2):350–4. https://doi.org/10.1016/j.vaccine.2019.10.005.
[8] Skowronski DM, Zou M, Clarke Q, Chambers C, Dickinson JA, Sabaiduc S, et al. Influenza vaccine does not increase the risk of coronavirus or other noninfluenza respiratory viruses: retrospective analysis from Canada, 2010–2011 to 2016–2017. Clin Infect Dis 2020;71(16):2285–8. https://doi.org/10.1093/cid/ciaa626.
[9] Horns F, Dekker CL, Quake SR. Memory B cell activation, broad anti-influenza antibodies, and bystander activation revealed by single-cell transcriptomics. Cell Rep 2020;30(3):905–913.e6. https://doi.org/10.1016/j.celrep.2019.12.063.
[10] Loeb M, Russell ML, Moss L, Fonseca K, Fox J, Earn DJD, et al. Effect of influenza vaccination of children on infection rates in Hutterite communities: a randomized trial. JAMA – J Am Med Assoc 2010;303(10):943. https://doi.org/10.1001/jama.2010.250.
[11] Amanat F, Stadlbauer D, Strohmeier S, Nguyen THO, Chromikova V, McMahon M, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. Nat Med 2020;26(7):1033–6. https://doi.org/10.1038/s41591-020-0913-5.
[12] Ang JC, Wang B, Wang JJF, Zeng PYF, Krammer F, Ward BJ, et al. Comparative immunogenicity of the 2014–2015 Northern Hemisphere trivalent IIV and LAIV against influenza a viruses in children. Vaccines 2019;7(3):87. https://doi.org/10.3390/vaccines7030087.
[13] Fink G, Orlova-Fink N, Schindler T, Grisi S, Ferrer APS, Daubenberger C, et al. Inactivated trivalent influenza vaccination is associated with lower mortality among patients with COVID-19 in Brazil. BMJ Evidence-Based Med 2021;26(4):192–3. https://doi.org/10.1136/bmjebm-2020-111549.
[14] Massoudi N, Mohit B. Influenza vaccine and incidence of COVID-19 among healthcare workers. J Clin Immunol 2021;41(2):324–34. https://doi.org/10.1007/s10875-020-00925-0.
[15] Patwardhan A, Ohler A. The flu vaccination may have a protective effect on the course of COVID-19 in the pediatric population: when does severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Meet influenza? Cureus 2021;13. https://doi.org/10.7759/cureus.12533.
[16] Belingheri M, Paladino ME, Latocca R, De Vito G, Riva MA. Association between seasonal flu vaccination and COVID-19 among healthcare workers. Occup Med (Lond) 2020;70:665–71. https://doi.org/10.1093/occmed/kqaa197.
[17] Amato M, Werba JP, Frigerio B, Coggi D, Sansaro D, Ravani A, et al. Relationship between influenza vaccination coverage rate and COVID-19 outbreak: an italian ecological study. Vaccines 2020;8(3):535. https://doi.org/10.3390/vaccines8030535.
[18] Green I, Ashkenazi S, Merzon E, Vinker S, Golan-Cohen A. The association of previous influenza vaccination and coronavirus disease-2019. Hum Vaccin Immunother 2021;17(7):2169–75. https://doi.org/10.1080/21645515.2020.1852010.
[19] Jehi L, Ji X, Milinovich A, Erzurum S, Rubin BP, Gordon S, et al. Individualizing risk prediction for positive coronavirus disease 2019 testing: results from 11,672 patients. Chest 2020;158(4):1364–75. https://doi.org/10.1016/j.chest.2020.05.580.
[20] Epidemiological evidence for association between higher influenza vaccine uptake in the elderly and lower COVID-19 deaths in Italy, 2020. https://doi.org/10.1002/jmv.26120.

[21] Kowalska M, Niewiadomska E, Barański K, Kaleta-Pilarska A, Brożek G, Zejda JE. Association between influenza vaccination and positive sars-cov-2 igg and igm tests in the general population of Katowice region, Poland. Vaccines 2021;9:415. https://doi.org/10.3390/vaccines9050415.

[22] Caban-Martinez AJ, Santiago KM, Louzado-Feliciano P, Gonzalez M, Brotons A, Schaefer Solle N, et al. Influenza vaccination coverage and (SARS-CoV-2) seroprevalance in a fire department. Ann Epidemiol 2020;52:115. https://doi.org/10.1016/j.annepidem.2020.08.058.

[23] Kim T-S, Shin E-C. The activation of bystander CD8+ T cells and their roles in viral infection. Exp Mol Med 2019;51(12):1–9. https://doi.org/10.1038/s12276-019-0316-1.

[24] Bernasconi NL, Traggiai E, Lanzavecchia A. Maintenance of serological memory by polyclonal activation of human memory B cells. Science (80-) 2002;298 (5601):2199–202. https://doi.org/10.1126/science:1076071.

[25] Long Q-X, Tang X-J, Shi Q-L, Li Q, Deng H-J, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med 2020;26(8):1200–4. https://doi.org/10.1038/s41591-020-0965-6.

[26] Johansson MA, Quandelacy TM, Kada S, Prasad PV, Steele M, Brooks JT, et al. SARS-CoV-2 transmission from people without COVID-19 symptoms. JAMA Netw Open 2021;4(1):e2035057. https://doi.org/10.1001/jamanetworkopen.2020.35057.

[27] Han MS, Choi EH, Chang SH, Jin B-L, Lee EJ, Kim BN, et al. Clinical characteristics and viral RNA detection in children with coronavirus disease 2019 in the Republic of Korea. JAMA Pediatr 2021;175(1):73. https://doi.org/10.1001/jamapediatrics.2020.3988.