

# Relative Antibody Mediated Protection against Influenza in Vaccinated Children

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## ABSTRACT

**Background:** Adjuvanted influenza vaccine has been shown to elicit more robust vaccine immunogenicity, as measured by the Hemagglutination Inhibition (HAI) assay. Post-vaccination HAI titers are the accepted correlate of protection against influenza. However, the proportion of relative vaccine protection in adjuvanted vaccines that is mediated by increased HAI titers has not been investigated.

**Methods:** A cluster randomized controlled trial of influenza vaccines in Canadian Hutterite colonies was conducted from the 2016-2017 to the 2018-2019 influenza season. Children were vaccinated with either Quadrivalent Inactivated Influenza Vaccine (QIV), or the MF59 adjuvanted trivalent influenza vaccine (aTIV). Sera were collected prior to and four weeks after vaccination, and HAI titers against A/H3N2 vaccine antigens were measured. We tested for differences in the hazard rate of influenza A/H3N2 infection using Cox proportional hazards models, and the proportion of relative vaccine protection in adjuvanted vaccinees mediated by higher HAI titers was estimated using a causal mediation model.

**Results:** Antibody titers from 542 paired serum samples were available, with 32 laboratory-confirmed in-

fluenza infections. Most infections were due to A/H3N2 and occurred during a season of antigenic mismatch in the vaccine. Of 22 A/H3N2 infections, two occurred in the aTIV group (0.8%), versus 20 in the QIV group (6.6%). We estimated the relative adjuvanted vaccine efficacy against A/H3N2 as 87.8% (95% CI: 43.6%, 97.4%). The rise in post-vaccination titers in adjuvanted vaccinees explained 9.0% of vaccine protection, relative to quadrivalent controls.

**Conclusion:** Relative vaccine protection in adjuvanted vaccinees is not conclusively mediated by the superior induction of HAI titers. Adjuvanted vaccine effectiveness may be attributable to other mechanisms, such as increased engagement of the innate- or cell-mediated immune repertoire. As most cases occurred during an antigenically mismatched season, adjuvanted vaccine protection against heterologous strains of influenza may be driven by cellular immunity, and further work is required.

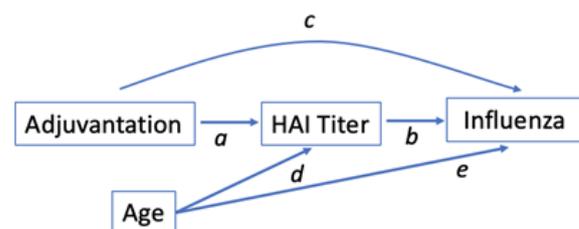
**Keywords:** Influenza, Adjuvanted influenza vaccine, Vaccination, Hemagglutination inhibition assay titers

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## INTRODUCTION

Seasonal influenza Vaccine Efficacy (VE) is measured by the robustness of hemagglutination antibody (HAI) titers, which have been shown to correlate with protection against infection (Hobson DR, *et al.*, 1972). Vaccination influences numerous immune mechanisms, including antibody production, and the activation of dendritic cells and T-cells, which in turn mediate the outcome of influenza infection. Previous work has found that HAI titers account for the majority (57%) of vaccine induced protection against influenza B, when comparing seasonal Trivalent Inactivated Influenza Vaccine (TIV) with placebo (Cowling BJ, *et al.*, 2019). The Adjuvanted seasonal influenza vaccine (aTIV) influences immune responses *via* numerous pathways, leading to enhanced protection (Vesikari T, *et al.*, 2011; Knuf M, *et al.*, 2014; Vesikari T, *et al.*, 2018). Studies have shown that the MF59 adjuvant directly effects the induction of cytokines, such as interferon-gamma and tumor necrosis factor, indicating that it is able to activate immune cells and enhance antigen uptake (O'hagan DT, *et al.*, 2012; Cioncada R, *et al.*, 2017). Other studies have demonstrated that it induces immunogenicity by the induction of chemokines, which increase the recruitment of immune cells such as neutrophils and monocytes; enhances differentiation of monocytes into dendritic cells; and facilitates dendritic cell migration into the lymph nodes, thus triggering an adaptive immune response (Zedda L, *et al.*, 2015; Khurana S, *et al.*, 2011). It is not surprising then that the MF59 adjuvant elicited significantly higher seroconversion rates and geometric mean titers against all vaccine antigens in children, as compared to TIV (Nolan T, *et al.*, 2014). Moreover, this study demonstrated that the persistence of HAI titers was significantly improved in adjuvant vaccinated children after six months,

suggesting that the adjuvant may increase magnitude and duration of protection. As discussed above, the MF59 adjuvant may mediate vaccine protection against influenza through its ability to induce higher post-vaccination HAI titers. However, to our knowledge no study has determined the proportion of adjuvant vaccine protection against influenza which is mediated by HAI titers. Our study used a causal mediation analysis to quantify the proportion of relative vaccine protection attributable to increased post-vaccination HAI titers in adjuvanted vaccinees, as compared to non-adjuvanted vaccines (Figure 1).



**Figure 1: Mediation pathway model between vaccination, adjuvant and associated HAI titers, and influenza infection status. Adjuvanted influenza vaccine indirectly affects the likelihood of influenza infection, as it induces a rise in HAI titers (a): Which correlates with reduced risk of flu; (b): Adjuvantation may also act *via* other mechanisms to directly induce protection, such as enhancing cell-mediated immunity (c) (O'hagan DT, *et al.*, 2012). Age has been shown to affect the magnitude of HAI titers which are induced in response to vaccination (d), and also the risk of influenza infection**

## MATERIALS AND METHODS

### Setting and study design

We conducted a cohort study, which made use of serum samples from the Adjuvanted Inactivated Vaccine versus Inactivated Influenza Vaccine in Hutterite Children Trial (NCT02871206). In the original cluster randomized controlled trial, 994 children aged 6 to 72 months were allocated to receive either quadrivalent inactivated influenza vaccine (QIV, Sanofi Pasteur: Fluzone<sup>®</sup>), or adjuvanted trivalent influenza vaccine (aTIV, Seqirus: Fluad Pediatric<sup>™</sup>). The parent study was conducted over three influenza seasons (2016-2019), and vaccines contained the recommended antigens for each season (Supplementary Table 1). Participants were then followed for the duration of the influenza season for any signs or symptoms of respiratory infection. Participants reporting two or more symptoms were sampled by nasopharyngeal swab for a multiplex respiratory pathogen panel and viral genotyping. Influenza infections were confirmed by reverse-transcriptase Polymerase Chain Reaction (PCR).

### Laboratory testing

Our cohort included children in whom there were data available on post-vaccination HAI titers. In the parent study, sera were collected from study-vaccinated children at baseline and four weeks post-vaccination. Serum samples were tested by HAI assay against the four vaccine antigens recommended for each study year, per the standard protocol (13). In brief, samples were plated in serial twofold dilutions from an initial dilution of 1:10, and incubated with erythrocytes and a stock titer of each vaccine strain virus. HAI titers were determined to be the reciprocal of the last dilution at which hemagglutination was inhibited. Titers of <10 were imputed at 5 for the analysis.

### Statistical analysis plan

The primary outcome of our analysis was time to influenza A infection, as confirmed by PCR. We hypothesized a mediation model (Figure 1), wherein adjuvanted influenza vaccination impacted HAI titers, as well as inducing other immune mechanisms against influenza infection. The adjuvant vaccine-induced protection against infection was mediated by the rise in HAI titers generated by a robust response to the vaccine (the mediating, “indirect” effect). Since age has been shown to affect the magnitude of HAI responses in children, as well as their likelihood of developing an influenza infection, we included this as a potential confounder in our models (Davenport FM, *et al.*, 1953; Ranjeva S, *et al.*, 2019).

### Estimation of total effect

To estimate the total effect of vaccination on protection, we constructed a Cox proportional hazards model, where the independent variables were the vaccine allocation (QIV or aTIV) and age, and the dependent variable was time of PCR-confirmed influenza infection. We included a term for the colony (the clustered variable) to estimate cluster-robust variance. The relative vaccine efficacy was calculated as  $1 - \text{the hazard ratio (HR)} \times 100\%$ . We tested whether there was an association between the adjuvant-induced rise in post-vaccination HAI titers and the hazard of influenza infection by fitting a Cox proportional hazards model, with age and vaccine allocation as the predictors, and a cluster-robust estimation term for colony. We determined whether there was an interaction between vaccine formulation and the post-vaccination titers by adding an interaction term to the model.

### Estimation of direct effect: Relative vaccine-induced protection

We estimated the direct effect of adjuvanted vaccination relative to quadrivalent vaccination on protection against influenza (the effect of the vaccine which does not act through the pathway of increased HAI titers) by first fitting a logistic regression model. We regressed the probability of vac-

ination with aTIV as the outcome onto post-vaccination HAI titers and age as predictors, adjusting for colony. The regression coefficients of this model were used to generate odds ratios for each child, from which we derived a weighted score for each vaccinated participant (1-OR) (WHO, 2011; Tchetgen TEJ, 2013). Weights for children receiving the QIV vaccine were pre-specified at 1. We then fit a proportional hazards model for the time to influenza infection, adjusting for age, vaccine group, and colony, weighted according to the scores generated by the odds ratios. The direct effect was estimated from the hazard ratio of this weighted model.

### Estimation of indirect effect: HAI-mediated relative vaccine-induced protection

The indirect effect was calculated as the ratio between the total effect and the direct effect (steps described above). The proportion of effect which is attributed to the HAI titres following was estimated as  $(\log(\text{indirect effect HR}) / (\log(\text{total effect HR})))$ . We reported the estimates, 95% confidence intervals, and p-values from the models for total and direct effects. We conducted a sensitivity analysis in which we adjusted for pre-vaccination titers, to account for the possibility that vaccination would not have elicited a robust immune response due to a ceiling-effect for antibody titres in children with previously high titers. Models followed the same approach outlined above, with pre-vaccination titers for each participant included as a covariate. We included only samples with complete data on pre-and post-vaccination titers; due to study design, there were no observations with missing data. All analyses were done in the R environment, version 4.0.2, and an alpha of 0.05 was considered significant for all tests (GBIF, 2020).

## RESULTS

Our cohort included 542 post-vaccination serum samples from 330 unique children across the three influenza seasons of the original study (Table 1). The mean age was 54.4 months (SD: 17.9) and did not differ significantly across vaccine groups. Among these children, there were 32 PCR-confirmed influenza infections: One A/H1N1 case, 22 cases of A/H3N2, and 9 B infections (untyped). Given the small number of cases in the available samples, and the significant differences between vaccine groups in post-vaccination titers against A/H3N2, we focus on infections of this subtype. Of 22 A/H3N2 infections, two occurred in the aTIV group (0.8%), as compared to 20 in the QIV group (6.6%) ( $X^2=9.97$ , 95% CI: -0.09, -0.02,  $p<0.001$ ). Given the small number of events, and that 82% of cases occurred in season two, we pooled cases across all seasons. The post-vaccination HAI titers against A/H3N2 are shown in Figure 2, and Table 2. Of 542 observations, 239 were in aTIV recipients, and 303 in QIV recipients. The geometric mean HAI titers were 355 (SD: 3.49) in aTIV vaccinees, as compared to 202 (SD: 5.07) in QIV vaccinees ( $p<0.001$ ). Pre-vaccination titers did not differ significantly ( $68.80 \pm 7.00$  in aTIV vaccinees, vs.  $54.22 \pm 6.76$  in QIV vaccinees,  $p=0.15$ ). The distribution of titers in all children are shown in Figure 3A, and the distribution of post-vaccination titers in children who went on to develop flu infections in Figure 3B. We estimated the hazard of influenza A/H3N2 infection in children who received the adjuvanted vaccine, as compared to QIV. We estimated the total effect HR to be 0.122 (95% CI: 0.026, 0.565), corresponding to a relative vaccine efficacy of 87.8% (95% CI: 43.6%, 97.4%). Under our proposed causal framework, we estimated that the direct effect, or the amount of vaccine protection which was not mediated by the increase in post-vaccination HAI titers, was 0.148 (95% CI: 0.03, 0.706); the indirect effect was estimated at 0.827. Using the ratio between the estimated log HRs for indirect and total effects, we estimated the proportion of relative vaccine effect mediated by the rise in higher antibody titers in adjuvant vaccinated children as 9.02%. There was no interaction observed between the post-vaccination titers and the vaccine formulation ( $p=0.576$ ).

Table 1: Characteristics of children with paired serum samples included in the study cohort

Demographics	All children (n=542)		aTIV (n=239)		QIV (n=303)	
	n	%	n	%	n	%
Age (months) (mean, SD)	54.4	17.9	54.8	18	54.11	17.8
Sex (Male=1)	286	52.8	126	52.7	160	52.8
PCR-confirmed influenza	32	5.9	7	2.9	25	8.3
A type	23	4.2	3	1.3	20	6.6
A/H1N1	1	0.2	0	0	1	0.4
A/H3N2	22	4.1	2	0.8	20	6.6
B type	9	1.7	4	1.7	5	1.7

Note: Where n=542 observations from 330 unique children

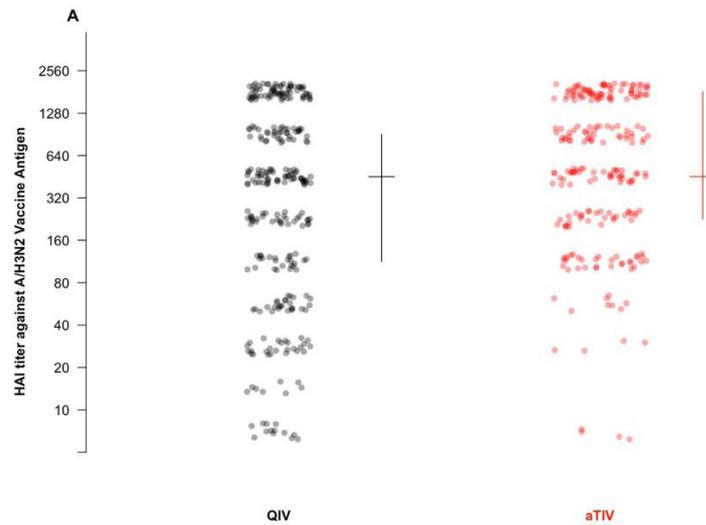


Figure 2: Distribution of post-vaccination HAI titers against the vaccine antigen for influenza A/H3N2  
 Note: Where black colour=Quadrivalent vaccinees and red colour=Adjuvanted vaccines; Vertical lines denote the interquartile range of titer values.

Table 2: Geometric mean pre-and post-vaccination HAI titers (mean, SD) and significance of difference between vaccine groups (unadjusted)

Antigen	Pre-vaccination titers			p	Post-vaccination titers			p
	All	aTIV	QIV		All	aTIV	QIV	
A/ H1N1	56.21	80.93	42.16	<0.001	211.72	478.88	111.21	<0.001
	7.27	6.71	7.39		5.53	3.21	6.03	
A/ H3N2	60.23	68.8	54.22	0.155	259.12	355.22	202.05	<0.001
	6.87	7	6.76		4.46	3.49	5.07	
B/ Victoria	17.2	19.95	15.3	0.04	111.99	120.42	105.75	0.323
	4.41	4.49	4.31		4.56	4.54	4.58	
B/ Yamagata	25.85	26.73	25.17	0.667	148.37	122.89	172.15	0.014
	5	4.97	5.04		4.84	5.07	4.59	

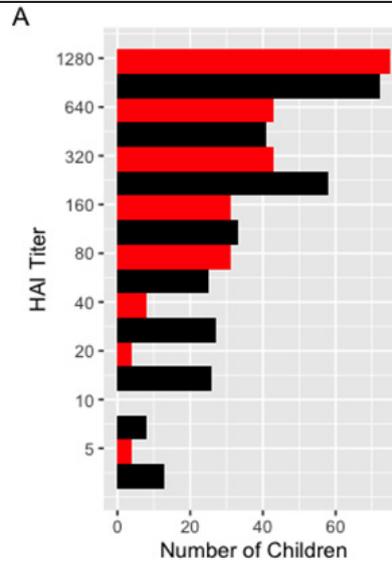


Figure 3A: Histogram of post-vaccination HAI titers, by vaccine allocation

Note: Where quadrivalent vaccinees are presented in black and adjuvanted vaccinees in red. Bars represent the count of children with HAI titers in each range of titer dilutions, shown as the reciprocal of the titer value (ranging from <10 to 1280).

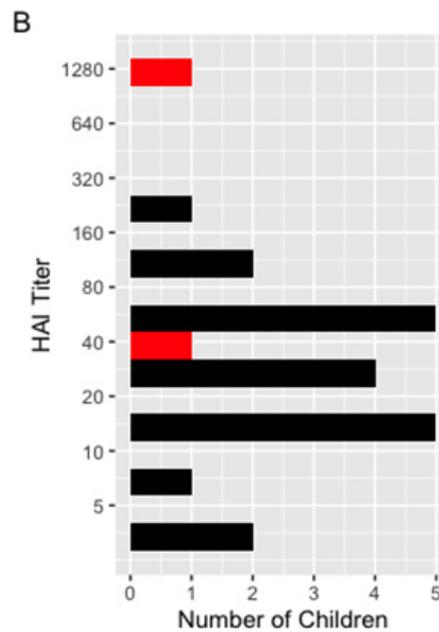


Figure 3B: Histogram of post-vaccination HAI titers in children who went on to develop influenza A/H3N2 infection, by vaccine allocation

Note: Where quadrivalent vaccinees are presented in black and adjuvanted vaccinees in red. Bars represent the count of children with HAI titers in each range of titer dilutions, shown as the reciprocal of the titer value (ranging from <10 to 1280).

We conducted a sensitivity analysis to account for pre-vaccination HAI titers, and the potential antibody ceiling effect, which might impact the magnitude of post-vaccination titer increases or fold-change. In QIV vaccinated children, 21% of children had no change between pre- and post-vaccination titers. Similarly, 18% of aTIV vaccinees had identical titers between time points. In our sensitivity model including pre-vaccination titers for each child, the total effect was estimated at 0.1305 (95% CI: 0.032, 0.532). The direct effect was 0.129 (95% CI: 0.032, 0.519). The indirect effect was estimated at 1.01%, suggesting no mediation as shown in Figure 4, the predicted hazard of infection in aTIV vaccinees relative to QIV vaccines declined well before the accepted correlate of influenza

protection HAI titer of 1:40. To estimate a relative risk reduction of 50%, we conducted a post-hoc analysis of the predicted risk scores at each titer dilution. We tested for a difference in the mean risk scores at each titer value by vaccine group using Welch's two-sample t-test, assuming unequal variance. Significant differences in the mean predicted risk scores were observed at every dilution ( $p < 0.05$  for all outcomes) (Table 3). Differences in risk scores by vaccine group where tested in each stratum of post-vaccination titers using Welch's two sample t-test, assuming unequal variance. Of note, we observed that relative to QIV vaccinees, children in the aTIV group were predicted to have a risk reduction of 50% or greater, regardless of the post-vaccination titer (Figure 5).

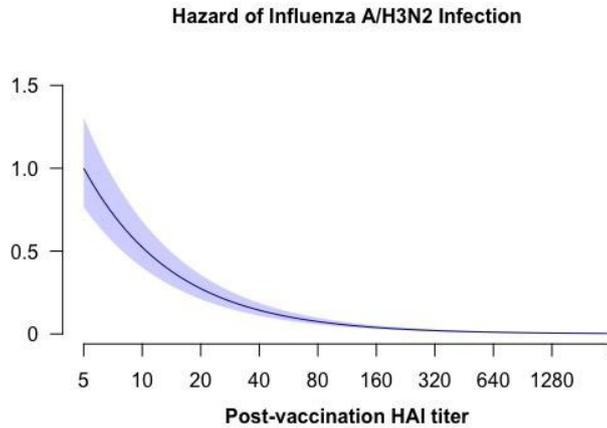


Figure 4: Correlation of HAI titer with protection against influenza A/H3N2 infection in a proportional hazards model. Assuming that the baseline risk is equal to one, the increase in post-vaccination HAI titer demonstrates relative risk reduction as HAI titers increase. Where the relative risk in quadrivalent vaccinees is one, this figure shows that adjuvanted vaccinees are at a reduced risk of influenza A/H3N2 infection at any value of HAI titer. An HAI titer of 40 is accepted to correlate with 50% protection against influenza; here we show that adjuvanted vaccinees are at 50% reduced risk at titers of less than 40 (add this line in the content)

Table 3: Mean predicted risk scores (SD) of post-vaccination titers, after adjustment for vaccine, age and colony

HAI titer	n (%)	Predicted risk score (mean, SD)			95% CI	p
		All children	aTIV	QIV		
5	17 (3.1%)	1.97 (0.94)	0.37 (0.01)	2.47 (0.22)	1.96, 2.23	<0.001
10	8 (1.5%)	2.45 (0.19)	-	2.45 (0.19)	-	-
20	30 (5.5%)	2.18 (0.74)	0.37 (0.04)	2.45 (0.19)	2.00, 2.17	<0.001
40	35 (6.5%)	2.08 (0.96)	0.38 (0.03)	2.59 (0.21)	2.13, 2.30	<0.001
80	56 (10.3%)	1.38 (1.13)	0.38 (0.03)	2.62 (0.20)	2.16, 2.32	<0.001
160	64 (11.8%)	1.54 (1.15)	0.38 (0.03)	2.64 (0.22)	2.18, 2.34	<0.001
320	101 (18.6%)	1.68 (1.12)	0.39 (0.03)	2.63 (0.18)	2.19, 2.29	<0.001
640	84 (15.5%)	1.46 (1.11)	0.39 (0.03)	2.58 (0.18)	2.13, 2.25	<0.001
1280	147 (27.1%)	1.50 (1.14)	0.39 (0.03)	2.65 (0.19)	2.22, 2.31	<0.001

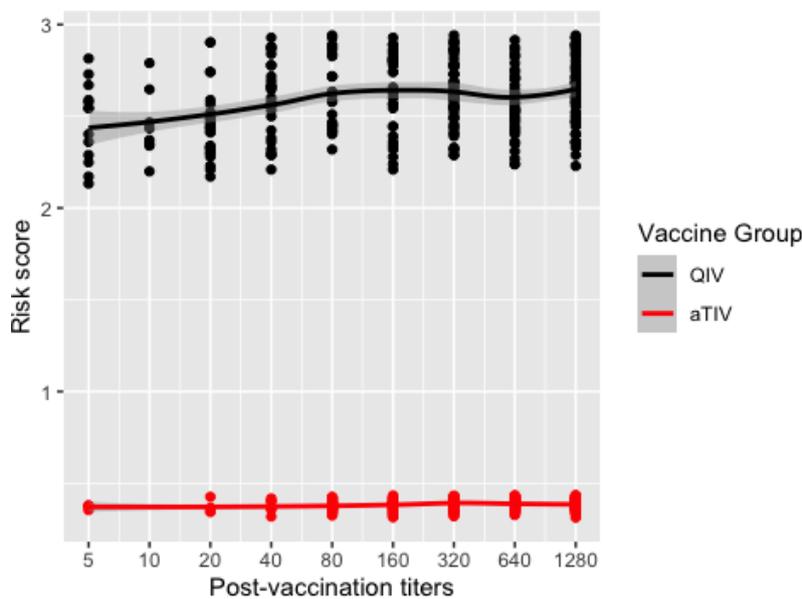


Figure 5: Predicted risk of influenza A/H3N2 infection from a proportional hazards model. In this model, risk of infection is predicted from the values of covariates (age, vaccine group) and weighted by the post-vaccination titer  
 Note: Red colour=adjuvanted vaccinees are less than half as likely to develop influenza A/H3N2, regardless of HAI titer.

## DISCUSSION

Our study evaluated the proportion of relative vaccine protection which is conferred by in rise in post-vaccination HAI titers induced by adjuvanted influenza vaccines. The MF59 adjuvant has been shown to increase flu vaccine immunogenicity and efficacy (Vesikari T, *et al.*, 2009). Despite the observed changes in vaccine effectiveness, the mechanism by which MF59 acts to enhance protection remains unclear (O'hagan DT, *et al.*, 2012). The HAI titer of <1:40 is the accepted correlate of protection against influenza, by which vaccine efficacy is assessed (FDA, 2017). In our study, we observed that the additional vaccine protection afforded by adjuvanted vaccinees was not largely driven by the greater HAI titers in this group. This finding offers interesting insight on both the utility of the HAI titer as a correlate of protection in children, and the adjuvant effect of MF59 on vaccine efficacy. Our finding suggests that post-vaccination HAI titers mediate little of the relative protection of adjuvanted influenza vaccine, an estimated 9.0%. Despite non-significant mediation attributable to HAI responses, adjuvanted vaccinees had significantly improved vaccine efficacy against A/H3N2. This suggests that the MF59 adjuvant, while it does induce stronger antibody responses to vaccination, does not improve protection *via* this pathway. Studies in children have shown that seroprotection evoked by MF59-adjuvanted vaccines in children is superior to the immune responses following nonadjuvanted vaccines (Zedda L, *et al.*, 2015; Nolan T, *et al.*, 2014; Vesikari T, *et al.*, 2015). However, studies evaluating the HAI threshold of <1:40 in children have found that it was not consistently correlated with protection against influenza, with new thresholds proposed of <1:110 (Black S, *et al.*, 2011; Ng S, *et al.*, 2019). Our study found that while immunogenicity was significantly enhanced in adjuvanted vaccinees, the relative protection of the adjuvanted vaccine was not conclusively mediated by the rise in HAI titers. This would suggest that MF59 adjuvant may confer improved relative vaccine efficacy *via* alternate pathways, such induction of innate-and cell-mediated immune responses. To our knowledge, our study is the first to use a causal mediation framework to assess relative vaccine protection. Cowling BJ, *et al.*, 2019 used a similar framework to model the proportion of vaccine protection against influenza B that was mediated by HAI titers, as compared to unvaccinated children. This study found that post-vaccination HAI titers mediated 57% of an overall vaccine efficacy of 68% against influenza B. Strengths of this work included the use of an unvaccinated comparator group, and its randomized controlled study design. In contrast, our study assessed the relative protection of adjuvanted versus non-adjuvanted influenza vaccines against influenza A/H3N2. Given that all study participants were vaccinated and mounted HAI titers, we would expect to see a more modest effect than might have been observed if we had compared with an unvaccinated control group. Our study also differs in that it investigates influenza A/H3N2, rather than B. Both antibody- and cell-mediated immune responses have been implicated in adaptive immunity against influenza A types (Sridhar S, *et al.*, 2015; Eickhoff CS, *et al.*, 2019; Ellebedy AH, *et al.*, 2014). It is possible that the effect of HAI titers on vaccine-induced protection may differ according to influenza type. Further work to compare the relative proportion of protection mediated by antibody titers against influenza across types and subtypes would have valuable insight for novel vaccine development.

Studies have shown that early life exposures to influenza imprint the host immune system, influencing the immune responses and magnitude of protection against subsequent exposures (Davenport FM, *et al.*, 1953; Gostic KM, *et al.*, 2019; Dugan HL, *et al.*, 2020; Fonville JM, *et al.*, 2014; Nuñez IA, *et al.*, 2017). Effects can include blunting of the immune response and epitope-biased responses, wherein antibody titers are increased to the novel exposure but absolute titers to primary exposure strains remain highest due to back boosting (Fonville JM, *et al.*, 2014; Zarnitsyna VI, *et al.*, 2016). In our cohort, most influenza A/H3N2 cases occurred in

season two and the mean age of children enrolled during this season was 4.5 years. Hence, as suggested by the literature (Séblain GE, *et al.*, 2019), children in this season would have already experienced an exposure to influenza, likely A/H3N2. This may mean that a significant proportion of HAI titers mounted in response to vaccination were memory responses to a previously encountered strain, rather than the vaccine antigen. Therefore, it is very likely that primary A/H3N2 exposures in study subjects were to antigenically drifted strains of A/H3N2 in previous seasons, which had acquired mutations in antigenic site B (Chambers BS, *et al.*, 2015; Zost SJ, *et al.*, 2017). These prior immune histories may have influenced the HAI responses to study vaccines, potentially limiting the quality and appropriateness of HAI titers and subsequent protection conferred.

There is a growing body of work on the effect of repeated vaccination on immunogenicity and efficacy of influenza vaccines (Belongia EA, *et al.*, 2017; McLean HQ, *et al.*, 2016; Thompson MG, *et al.*, 2016; Vesikari T, *et al.*, 2020; Ramsay LC, *et al.*, 2019). A seminal modelling study by Smith DJ, *et al.*, 1999 proposed the antigenic distance hypothesis, which predicts that vaccine efficacy is reduced when the vaccine antigen is closely similar to the prior season antigen, but dissimilar to the circulating strain. In season two of our study, the vaccine antigen was unchanged from the previous season recommendation; however, phylogenetic analyses of A/H3N2 evolution showed rapid mutation and clade diversification from 2013-2018 (Chambers BS, *et al.*, 2015; Potter BI, *et al.*, 2019; Skowronski DM, *et al.*, 2022). Skowronski DM, *et al.*, 2017 found that prior influenza vaccination had mixed effects across seasons, consistent with the antigenic distance hypothesis. Prior seasonal vaccination was significantly associated with negative impact on current VE during the 2017-2018 season, in which vaccine antigens were homologous with the previous season's formulation but circulating strains had the greatest phylogenetic distance from the vaccine antigen (Skowronski DM, *et al.*, 2017). This would suggest that both viral evolution and changes in host immunity due to serial vaccination may contribute to poor vaccine effectiveness during season two of our study.

As a result of the potential interference by repeat vaccinations and the acquisition of mutations in A/H3N2 which may have impeded efficient antibody binding during season two, HAI titers to the vaccine antigen may not have been sufficiently cross-protective. Therefore, it is challenging to ascertain the true proportion of relative vaccine protection which is mediated by the adjuvant-induced rise in HAI titers, had the vaccine been better matched to the seasonal circulating strain. Further study is warranted to investigate the causal mediation of relative adjuvanted vaccine protection by HAI titers during a matched influenza vaccine season. Our study has several other limitations, including the very small number of PCR-confirmed influenza cases, particularly in the adjuvanted group. It is possible that the low proportion of effect mediated by the rise in HAI titers in this group is underestimated due to the small sample size. Among confirmed influenza cases, HAI titers were not available for all children, further reducing our number of events and decreasing statistical power.

## CONCLUSION

Our study provides unique insight on the potential mechanism of adjuvant-induced influenza vaccine protection. We found that while adjuvantation results in significant differences in post-vaccination titers, and confers significantly superior protection against influenza A/H3N2 infections, the increased HAI titers do not conclusively mediate this protection in adjuvanted vaccinees. We observed that relative to QIV vaccinees, the hazard of A/H3N2 in adjuvanted children was reduced by 50% at titers >1:20. Possible explanations for this include adjuvant-induced innate or cell-mediated immune responses. Moreover, influenza infections in our study were from a season of co-circulating H3N2 strains from divergent clades. Despite the differing epidemic strains, protection was significantly better in the adjuvanted group relative to non-adjuvanted vaccinees.

This suggests that adjuvanted influenza vaccine may confer enhanced cross-protection against novel influenza antigens, potentially in seasons of vaccine mismatch. This would provide strategic data for the development of novel vaccine candidates against influenza A types, particularly A/H3N2, which shows consistently weaker vaccine effectiveness. Further research on the potential cross-protection conferred by adjuvanted influenza vaccine would be powerful for the design of next generation vaccines and adjuvant formulations.

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