Serial vaccination and the antigenic distance hypothesis: effects on influenza vaccine effectiveness during A(H3N2) epidemics in Canada, 2010-11 to 2014-15

Danuta M Skowronski[1,2], Catharine Chambers[1], Gaston De Serres[3,4,5], Suzana Sabaiduc[1], Anne-Luise Winter[6], James A Dickinson[7], Jonathan B Gubbay[6,8], Kevin Fonseca[9,10], Steven J Drews[11,12], Hugues Charest[3], Christine Martineau[3], Mel Krajden[1,2], Martin Petric[2], Nathalie Bastien[13], Yan Li[13], Derek J Smith[14]

Author affiliations:

1. British Columbia Centre for Disease Control, Vancouver, Canada
2. University of British Columbia, Vancouver, Canada
3. Institut National de Santé Publique du Québec (National Institute of Health of Quebec), Québec, Canada
4. Laval University, Quebec, Canada
5. Centre Hospitalier Universitaire de Québec (University Hospital Centre of Quebec), Québec, Canada
6. Public Health Ontario, Toronto, Canada
7. University of Calgary, Calgary, Canada
8. University of Toronto, Toronto, Canada
9. Alberta Provincial Laboratory, Calgary, Canada
10. University of Calgary, Calgary, Canada
11. Alberta Provincial Laboratory, Edmonton, Canada
12. University of Alberta, Edmonton, Canada
13. National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada
14. University of Cambridge, Cambridge, England

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A. ABSTRACT

Background—The antigenic distance hypothesis (ADH) predicts that negative interference from prior season's influenza vaccine (*v1*) on current season's vaccine (*v2*) protection may occur when the antigenic distance is small between *v1* and *v2* (*v1≈v2*) but large between *v1* and the current epidemic (*e*) strain (*v1≠e*).

Methods—Vaccine effectiveness (VE) against medically-attended, laboratory-confirmed influenza A(H3N2) illness was estimated by test-negative design during three A(H3N2) epidemics (2010-11, 2012-13, 2014-15) in Canada. VE was derived with covariate adjustment across *v2* and/or *v1* categories relative to no vaccine receipt among outpatients ≥9-years-old. Prior vaccination effects were interpreted within the ADH framework.

Results—Prior vaccination effects varied significantly by season, consistent with the ADH. There was no interference by *v1* in 2010-11 when *v1≠v2* and *v1≠e*, with comparable VE for *v2* alone or *v2+v1*: 34%(95%CI:-51,71) vs. 34%(95%CI:-5,58). Negative interference by *v1* was suggested in 2012-13 with non-significant reduction in VE when *v1≈v2* and *v1≠e*: 49% (95%CI:-47,83) vs. 28% (95%CI:-12,54). Negative effects of prior vaccination were pronounced and statistically significant in 2014-15 when *v1≡v2* and *v1≠e*: 65%(95%CI: 25,83) vs. -33%(95%CI: -78,1).

Conclusions—Effects of repeat influenza vaccination were consistent with the ADH and may have contributed to findings of low VE across recent A(H3N2) epidemics since 2010 in Canada.

Key words: influenza; influenza vaccine; vaccine effectiveness; influenza A(H3N2) subtype; repeat vaccination; antigenic distance hypothesis; negative interference; genomic sequencing; hemagglutination inhibition; antigenic site

Abbreviations:
AD=antigenic distance; ADH=Antigenic distance hypothesis; CI=confidence interval; *e*=epidemic strain; *v2*=current season's vaccine; *v1*=prior season's vaccine; *v0*=vaccine of two prior season's ago; TND=test-negative design; VE=vaccine effectiveness; ≈ means similar; ≡ means identical; ≠ means not similar

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B. BACKGROUND

A growing body of evidence suggests that protection from seasonal influenza vaccine may be modified by vaccination in prior seasons[1-13]. Hoskins et al were the first to report such effects during a series of three boarding school outbreaks due to influenza A(H3N2) in the 1970s[1-3]. Across the three outbreaks (1972, 1974, 1976), children who were repeatedly vaccinated, recently vaccinated only, or consistently unvaccinated experienced similar cumulative attack rates, leading authors to conclude that annual influenza vaccination conferred no long-term advantage[3]. In the context of vaccine that was at least partially protective during some outbreaks, however, the finding of comparable cumulative attack rates implies that during other outbreaks repeatedly vaccinated children were at increased risk. Indeed, during the final spring 1976 outbreak due to antigenically-drifted A/Victoria virus mismatched to the current A/Port Chalmers vaccine (the latter also used as vaccine antigen the prior season), repeatedly-vaccinated children had attack rates that were ~50% higher than consistently-unvaccinated children(**Supplement_1**)[3].

In a follow-up efficacy trial, Keitel et al examined the effects of annually re-administered trivalent influenza vaccine among non-elderly community-dwelling adults[4]. Across the five study seasons (1983-84 to 1987-88) authors found variable effects of repeat vaccination, widely interpreted to contradict Hoskins[1-4]. Both studies administered whole virus vaccines at doses that are no longer applicable and both included serologic diagnosis of influenza now recognized to over-estimate vaccine protection[1-4]. However, during the final 1987-88 study season, when the A(H3N2) vaccine component was closely related to the prior season's vaccine but distinct from the epidemic strain, and

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with restriction to include only randomized participants and virologically-confirmed outcomes, Keitel reported similar findings to Hoskins[3,4]. With more annual vaccinations there was significant 48% higher A(H3N2) risk. This pattern was not linear but was driven by rates of culture-confirmed infection that were 2.7-fold higher among maximally-vaccinated participants compared to placebo recipients(p=0.07)(**Supplement_2**). In combination, the Hoskins and Keitel studies signaled that repeated vaccination could be associated with reduced protection and increased influenza susceptibility under certain conditions in some seasons, but neither study was adequately powered to resolve the issue[3,4]. Subsequent meta-analysis concluded no evidence for decreasing protection with annually-repeated influenza vaccination; those conclusions, however, were reached with broad pooling across seasons, subtypes, settings, vaccines, age-groups and serological/virological outcomes[14].

In modeling simulations during the late 1990s, Smith et al attempted to reconcile variable observations of repeat influenza vaccination effects through a unifying antigenic distance hypothesis (ADH)[15]. The ADH assigned ADs between vaccine and epidemic strains based on the hemagglutination inhibition (HI) assay[16], derived as $\log_2$ of the fold-difference in HI antibody titres between homologous and heterologous comparator strains. This was translated into a predictive mathematical model for relative vaccine effectiveness (VE) but without absolute clinical meaning[15]. In this model, repeat vaccination effects were foremost determined by the AD between prior (*v1*) and current (*v2*) season's vaccines and between *v1* and the current season's epidemic (*e*) strain[15].

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According to the underlying theory of associative memory, prior vaccination effects represent a balance between pre-existing *v1*-induced antibody potentially interfering with *v2* antigen, and *v2* stimulation of rapid *v1* memory responses potentially protective against *e*. When *v1* and *v2* are more antigenically distinct (i.e. $v1 \neq v2$), their interactions should be minimal. Conversely, when the AD between *v1* and *v2* is smaller (i.e. $v1 \approx v2$), effect modification by *v1* on current season's VE becomes more likely. Negative interference is anticipated when $v1 \approx v2$ but the AD between *v1* and *e* is large (i.e. $v1 \neq e$). Pronounced negative effects from *v1* on VE are anticipated under the extreme scenario of homologous (i.e. identical) vaccine components in the current and prior season (i.e. $v1 \equiv v2$), and $v1 \neq e$. By comparison, positive interference is anticipated when the AD between *v1* and *e* is smaller (i.e. $v1 \approx e$).

Since the 2004-05 season, the test-negative design (TND) has been used globally to monitor influenza VE annually[17]. A recent meta-analysis of TND studies (>90% published since 2010) highlighted low VE (<40%, on average) for the A(H3N2) subtype[17]. This low VE was not well-explained by current season's vaccine match to the circulating strain (i.e. *v2-e* relatedness); accordingly other explanatory agent-host factors have been sought, including the ADH[5,7-13]. The Canadian Sentinel Practitioner Surveillance Network (SPSN) is unique in linking prior and current season's vaccine history to detailed genetic characterization of influenza variants collected from VE study participants[8-10]. Here we use the clinical and virological databases of this integrated platform to explore effects of prior vaccination on current season's VE during recent A(H3N2) epidemics in Canada since 2010-11. Findings are interpreted within the ADH

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framework primarily invoking *v1*, *v2* and *e* relatedness, with secondary consideration also of an additional prior season's vaccine (*v0)* receipt.

C. METHODS

1. Canadian SPSN

Patients presenting within 7days of influenza-like illness (ILI) onset to outpatient sentinel clinics in participating provinces (Alberta/British Columbia/Ontario/Quebec) were eligible. ILI was defined as acute respiratory illness requiring fever and cough and at least one of sore throat, arthralgia, myalgia, or prostration. Fever was not a requirement in patients ≥65-years-old. Influenza was diagnosed by RT-PCR at provincial reference laboratories from specimens collected by nasal/nasopharyngeal swab. Epidemiological data, including receipt of current (*v2*) and up to two previous seasons' sequential vaccines (*v1* and *v0*), were collected by sentinel practitioners from consenting patients/guardians using a standard questionnaire at specimen collection, before laboratory testing.

2. Analysis of current and prior vaccination effects

Patients testing positive for influenza A(H3N2) were considered cases, while those testing negative for any influenza were considered controls. The odds ratio (OR) for medically-attended, laboratory-confirmed influenza A(H3N2) illness was derived by logistic regression across self-reported vaccination categories using an indicator variable: i)unvaccinated both current and prior season (reference group), ii)vaccinated prior but not current season, iii)vaccinated current but not prior season and iv)vaccinated both current

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and prior season. VE was derived as (1–OR)*100%. ORs in relation to current but not prior season vaccination as the reference group were also assessed.

Only seasons for which the A(H3N2) subtype comprised the large majority of influenza A detections were included: 2010-11 (80% of detections)[8], 2012-13 (81% of detections)[9] and 2014-15 (97% of detections)[10]. The analysis period spanned November 1—April 30 each season. Participants reporting current season's vaccination <2weeks before ILI onset were excluded. For consistency in age-based dosing recommendations, participants<9-years-old were also excluded. Adjustment for the same potential confounders was applied each season including age-group(9-19/20-49/50-64/≥65-years-old); sex(female/male); comorbidity(no/yes); province(Alberta/British Columbia/Ontario/Quebec); interval from ILI onset to specimen collection(0-4/5-7days); and calendar-time(specimen collection week modeled using cubic B-spline functions with 3 equally-spaced knots). Participants missing vaccination status for the current and/or prior season or covariate information were excluded. Ethics review boards in each province provided study approval.

3. Influenza vaccines

Influenza vaccines were administered during the regular campaign commencing in October/November, offered without charge to all residents of Ontario/Alberta and to high-risk groups and their close contacts in British Columbia/Quebec. Vaccines were almost entirely trivalent non-adjuvanted inactivated products of which more than two-thirds were split virion and the remainder subunit. Adjuvanted and live-attenuated

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influenza vaccines were also available but primarily for groups excluded from this analysis.

4. Antigenic and genetic characterization of vaccine-virus relatedness

Sanger-sequencing of the viral HA1 gene in influenza test-positive specimens was undertaken each season to establish clade distribution and to detect notable amino-acid differences at established antigenic sites, labeled A-E for A(H3N2) viruses[8-10]. Genetic comparisons are between the dominant epidemic clade detected by the SPSN relative to the egg-adapted high-growth reassortant (HGR) vaccine used by manufacturers[8-10,18].

Antigenic relatedness across representative egg-passaged vaccine and cell-passaged epidemic reference viruses each season was quantified by the AD using HI titres posted by the WHO Collaborating Centre for Reference and Research on Influenza (London) as detailed in **Supplement_3**[15,19]. By convention, antigenic distinction of a heterologous strain is defined by ≥8-fold difference in HI-antibody titre relative to the homologous strain, corresponding to an AD≥3 (i.e. $\log_2 8=3$), although the Smith et al model allows for cross-reactivity between viruses up to ADs<7.

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C. RESULTS

1. Seasonal and participant profiles

A dominant A(H3N2) epidemic occurred during three of five seasons between 2010-11 and 2014-15(**Figure_1**), with considerable heterogeneity in the genetic and antigenic relatedness between *vo*, *v1*, *v2* and *e* strains(**Table_1)**.

Non-elderly adults 20-64-years-old comprised three-quarters of participants overall (2591/3477) and each season. Repeatedly vaccinated participants were significantly older (median 55 vs. 35-39years,p<0.01). All participants presented within 7days of illness onset but those vaccinated in the current season only more often presented later within that period compared to other vaccine groups (38% vs. 23-26% at 5-7days; median interval 4 vs. 3days,p<0.01)(**Table_2;Supplement_4**).

The proportion of test-negative controls overall who reported being vaccinated increased over time from 25%(207/813) in 2010-11 to 40%(382/951) in 2014-15. Among test-negative controls reporting current season's vaccination (*v2*), 84%(615/734) were also vaccinated the prior season (*v2+v1±v0*) and 77%(549/711) with complete information were vaccinated both prior seasons (*v2+v1+v0*). Among cases (but not controls) there was a substantial increase in the proportion reporting prior season(s)' vaccination in 2014-15(**Figure_2;Supplement_5**).

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3. VE: prior season(s)' effects stratified by season and vaccine-virus relatedness

The current season's vaccine was antigenically-distinct from the dominant circulating variant (i.e. *v2≠e*) for each epidemic, with *v2-e* ADs ranging 4-6(**Table_1;Supplement_3B**). Adjusted-VE did not exceed 40% during any epidemic but varied significantly by season (p<0.01 for season*current vaccine status interaction)(**Figure_3;Supplement_6**). Prior vaccination effects also varied significantly by season (p=0.01 for season*vaccine category interaction), precluding pooled analyses.

3a. 2010-11: *v1≠v2* (AD=4), *v1≠e* (AD=7)

There was no apparent interference by *v1* in 2010-11, and the interaction between *v1* and *v2* was not statistically significant. VE was comparable for recipients of *v2* alone and for *v2+v1*: 34%(95%CI=-51,71) vs. 34%(95%CI=-5,58), respectively(**Table_1;Figure_4;Supplement_7**). Those reporting *v1* alone were at significantly higher risk (VE=-55%;95%CI=-134,-3) compared to those unvaccinated both seasons. A role for *v0* (≡*v1;*AD=0) may be suggested by the higher VE point estimate among *v2* recipients who received neither *v1* nor *v0* (58%;95%CI=-32,87), compared to those receiving both (32%;95%CI=-11,58), although 95%CIs overlap(**Supplement_8**).

3b. 2012-13: *v1≈v2* (AD=1), *v1≠e* (AD=3)

For the 2012-13 season, HI characterization data were not available for the egg-passaged *v1* referent virus used by manufacturers (A/Victoria/210/2009); ADs were instead derived based on the egg-passaged version of the WHO-recommended *v1* referent

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(A/Perth/16/2009) which may not be suitably representative (potentially under-estimating ADs in relation to *v1*)(**Supplement_3B**). A pattern of negative interference by *v1* was evident in the higher point estimate of VE in 2012-13 for recipients of *v2* alone than *v2+v1*: 49%(95%CI=-47,83) vs. 28%(95%CI=-12,54) although 95%CIs overlap(**Table_1;Figure_4;Supplement_7**) and the interaction between *v1* and *v2* was not statistically significant. No added influence of *v0* (≡*v1;*AD=0) was apparent(**Supplement_8**). There was no residual protection from *v1* alone (VE=0%;95%CI=-66,39).

3c. 2014-15: *v1*≡*v2* (AD=0), *v1*≠*e* (AD=4)

For the 2014-15 season, only a small proportion of epidemic viruses could be successfully characterized by HI assay[20]. A glycosylation motif unique to the wild type epidemic strain was lost or partially lost with laboratory passage, potentially affecting HI characterization data and derived *v1*/*v2-e* ADs(Supplement_3B)[20]. Pronounced and statistically significant negative interference by *v1* on *v2* ($p<0.01$) was observed in 2014-15. VE was significantly higher for recipients of *v2* alone than *v2+v1*: 65%(95%CI=25,83) vs. -33%(95%CI=-78,1)(**Table_1;Figure_4;Supplement_7**). Increased risk among the repeatedly-vaccinated compared to the consistently-unvaccinated was significant with further consideration of *v0* (≈*v1;*AD=1) (OR=1.47;95%CI=1.08,2.01)(**Supplement_8; Supplement_9A**). Repeat vaccine recipients had significant four-fold higher odds of medically-attended A(H3N2) illness compared to those newly-vaccinated in 2014-

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15(**Supplement_7;Supplement_8;Supplement_9B**). There was no residual protection from *v1* alone (VE=-7%;95%CI=-59,28)(**Supplement_7;Figure_4**).

D. DISCUSSION

Using databases of the Canadian SPSN we explored the extent to which repeat vaccination effects may have contributed to suboptimal influenza vaccine performance during recent A(H3N2) epidemics in Canada. We interpret our findings within the framework of the ADH, comparing observed effects measured by the TND with predicted patterns based on the antigenic relatedness between prior season's vaccine (*v1*), current season's vaccine (*v2*) and the circulating epidemic strain (*e*). This is the first modern attempt to directly correlate AD metrics with epidemiological observations of *v1* effects and their overall fit within the ADH paradigm since it was first formulated nearly two decades ago.

Across the three A(H3N2) epidemics since 2010-11 in Canada, no adjusted seasonal VE estimate exceeded 40% even among mostly healthy, working-age adults. Each of these epidemics was associated with a vaccine-mismatched strain (*v2≠e*) although variation in VE was not obviously correlated with the AD (or match) between *v2* and *e*. Adjusted-VE was highest in 2010-11 (40%;95%CI=9,60), similar in 2012-13 (31%;95%CI=-4-55) but dramatically lower in 2014-15 (-12%;95%CI=-47,15) despite comparable *v2-e* ADs ranging 4-6. In original report of the ADH, Smith et al also highlighted a lack of correlation between VE and the *v2-e* distance in first-time vaccinees[15]. Since A(H3N2) epidemics are associated with the greatest influenza disease burden[21], understanding

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the agent-host factors that contribute to low VE is critical. Our findings suggest that prior vaccination may modify current VE and that this effect may vary by season according to the ADH. Given heterogeneity in the conditions of vaccine-virus relatedness, we should expect *v1* effects on current season's VE to vary by season. Pooling or averaging *v1* effects across seasons may enhance statistical power but at the risk of masking meaningful variation and insights to inform mechanisms and implications; further explorations of prior influenza vaccination effects should stratify results by season and subtype.

During the three A(H3N2) epidemics presented here, observed *v1* effects included no modification, as well as significant negative interference; we did not observe positive interference (i.e. boosting), also possible within the ADH framework but under specific conditions not found during epidemics included here[15]. In 2010-11, when *v1* and *v2* were antigenically distinct (*v1*≠*v2*) minimal or no interaction was expected or observed. Conversely, with closer but non-homologous *v1* and *v2* relatedness in 2012-13 (*v1*≈*v2*), the expected pattern of negative interference was apparent, although with limited sample size effect modification was not statistically significant. As anticipated based on the ADH, the negative effects of prior vaccination on current season's VE were most pronounced and statistically significant in 2014-15 with homologous *v1* and *v2* antigens (*v1*≡v2) and antigenically distinct circulating epidemic virus relative to *v1* (*v1*≠*e*).

Although antigenic drift has been widely emphasized to explain the historically-low VE in 2014-15, the AD between *v2* and *e* was not estimated to be dramatically different from

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recent prior seasons[10,11,22-24]. Conversely, prior vaccination had marked effects, negating the otherwise moderate VE observed among *v2*-only recipients despite vaccine mismatch. A similar pattern of moderate VE among *v2*-only recipients, substantially reduced with receipt of the prior season's homologous vaccine, was also reported for 2014-15 in multi-country analysis from Europe[11] but not from the US where VE against A(H3N2) was negligible in all categories of current and prior vaccine recipients[23]. In the Canadian data, a dramatic increase in the distribution of influenza A(H3N2) cases reporting prior vaccination was observed in 2014-15 whereas controls showed the expected trajectory of gradual increase, reflecting vaccine coverage trends in the general source population[25,26]. In all seasons, vaccination status was based on patient self-report and practitioner documentation before either knew the participant's case vs. control status (i.e. influenza test positivity result), minimizing differential recall bias and heightening the plausibility of the observation particular to cases in 2014-15.

In 2014-15 in Canada, under the specific conditions of $v0 \approx v1 \equiv v2 \neq e$, serial vaccination was associated with a nearly 50% increased risk of medically-attended A(H3N2) illness relative to participants who were consistently unvaccinated. Statistically significant increased risk (OR=1.85;95%CI=1.17, 2.90) of A(H3N2) illness in 2014-15 was also reported from Italy where vaccinated participants were also mostly repeat recipients[24]. The 2014-15 epidemic is the first season in more than a decade of annual VE monitoring for which the Canadian SPSN reported vaccine-associated increased risk, and caution is warranted in its interpretation. However, increased risk was previously reported by multiple studies from Canada and elsewhere during the 2009 A(H1N1)pdm09 pandemic

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in association with prior receipt of mismatched 2008-09 seasonal vaccine, replicated also in at least one randomized-controlled study in ferrets[27-31]. Influenza vaccine-associated enhanced respiratory disease (VAERD) is a well-recognized phenomenon following heterologous challenge in vaccinated swine, most of whom recover[32]. Although animal experiments may not be directly relevant to human experience, elements of involved mechanistic pathways may overlap and inform biological plausibility.

The ADH is a useful conceptualization but is not amenable to exact extrapolation[15]. The originally-published simulations were based on AD between *v2* and *e* set at 2 with variability explored around *v1-v2* and *v1-e*. Sensitivity analyses explored effects of homologous vaccination ranging up to a *v2-e* distance of 3, but not greater. Emphasis was placed on the most proximal prior vaccination; the effects of earlier or multiple prior virus or vaccine exposures were not considered. The ADH predicts relative, but not absolute VE and the possibility that serial vaccine receipt might be associated with increased risk under some conditions was not considered, although such signals may have already been evident in the studies by both Hoskins and Keitel under specific conditions of multiple repeat vaccinations and *v1*, *v2* and *e* relatedness[3,4](**Supplement_1;Supplement_2**). The ADH is predicated on the HI assay but variability in HI results by assay conditions must be acknowledged[16,20]. For example, in two of three epidemics analyzed here (2010-11, 2012-13) Canada's national influenza reference laboratory characterized all viruses as well-matched to the WHO-recommended *v2* reference strain (AD<3)[8,9,33,34]. Those characterizations, however, were in relation to the cell-passaged *v2* referent (whereas manufacturers use an egg-

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adapted reassortant), included varying animal-source erythocytes and did not include oseltamivir to address NA-mediated effects[8,9]. We based our AD calculations on HI assays standardized for these conditions by the WHO Collaborating Centre for Reference and Research on Influenza (London)[19]. Even so, further variability in the mix of variants by setting, the representativeness of selected reference strains, and changes induced by laboratory passaging complicates AD derivation, interpretation and generalization. Future evaluations and their extrapolation would benefit from the assembly of a standard and definitive library of HI characterizations and ADs between specific egg-passaged vaccine strains and circulating genetic variants, each season. The incorporation of modern genomic, bioinformatic mapping and antibody landscape approaches could also improve resolution in the understanding of vaccine-virus relatedness and response[35,36].

Vaccine effects beyond those involving the HA1 (i.e. HA2 or NA) and other agent-host immunological influences beyond (or complementary to) the ADH likely also play a role including possible heterosubtypic effects of trivalent vaccine not otherwise considered. Original priming (e.g. imprinting) and prominent recall (e.g. back-boosting) responses to historic influenza exposures can shape hierarchical antibody responses, with either positive or negative implications[37-42]. Annually-repeated vaccination, compared to less frequent infection exposures, may accelerate antibody re-focusing toward prior versus evolved epitopes, with selection for cross-reactive but non-neutralizing memory responses[43]. In the context of pre-existing antibody, immune complex formation and Fc-receptor activation can suppress B-cell response to subsequent influenza vaccine

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doses[44]. Antibody-dependent mechanisms may also suppress innate cytokine signaling pathways required for pro-inflammatory T-cell responses[45] and in children, annual repeat vaccination has been reported to hamper development of virus-specific CD8+ T cell immunity[46]. Repeat vaccination may also select for T-cell responses that are antagonistic, such as preferential activation and/or recruitment of regulatory cells upon re-exposure[47]. Such mechanisms may also modify risk in previous but not current vaccine recipients. Ultimately, the mechanisms to explain the potential negative effects of repeat vaccination remain unknown but are likely multi-factorial, requiring a more complex systems approach to resolve[48].

Random and systematic error, including residual confounding and behavioral differences, may also contribute to findings. Few A(H3N2) epidemics were analyzed here, and each season represented a unique set of specific vaccine-virus relatedness conditions. Sample size in our indicator-variable analyses was also limited. Additional seasons are required before definitive conclusions can be drawn about correlation with the ADH. Population-based immunization registries aren't available in Canada for the study period, but self-report is considered an accurate predictor of influenza vaccination status as demonstrated in US analyses relative to registry data for both current[49] and prior season's vaccination status[verbal communication, Ed Belongia Marshfield Clinic Research Foundation], especially among adults who comprise the majority (86%) of our participants. We have the greatest confidence in VE estimates for repeatedly-vaccinated relative to consistently-unvaccinated participants both in terms of reliable personal recall of vaccine history and also statistical certainty owing to sample size, but less confidence in smaller subsets of

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participants reporting more erratic vaccination behaviors. Change in vaccination habit may be correlated with influenza risk, a bias that has been raised previously in deriving VE estimates in elderly adults based on administrative datasets but also potentially relevant in assessing current/prior vaccination effects using an observational design[50]. First-time vaccinees may have been newly motivated to receive influenza vaccine because of recent acute respiratory illness, possibly due to influenza. In the context of recent prior infection, vaccine responses may be enhanced[51] and/or VE may be over-estimated through confounding by more durable and cross-protective infection-induced immunity. We did not have prior infection history, but the proportion of newly-vaccinated individuals with that recent history would have to be substantial to meaningfully influence VE estimates. Prior vaccination may have conversely blocked opportunity to acquire infection-induced immunity (i.e. infection-block hypothesis), leading to under-estimation of VE in the recurrently vaccinated—an indirect mechanism for repeat vaccination effects originally favored by Hoskins but insufficient to fully explain observed effects of vaccine-associated increased risk[3,27,31].

In summary, serial vaccination may have contributed to poor influenza vaccine performance during recent A(H3N2) epidemics in Canada. The ADH remains a useful framework for reconciling variability in repeat vaccination effects, but requires update to incorporate recent epidemiological findings; modern and standardized laboratory approaches for monitoring vaccine-virus relatedness and response; and a broader understanding of immunological context and consequences. Integrated immuno-epidemiological evaluation across an extended horizon is needed to understand the

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spectrum of repeat vaccination effects and to determine whether annual influenza vaccination is likely to provide long-term advantage at the individual or population levels—a return to the question first posed by Hoskins forty years ago[3].

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E. FOOTNOTE PAGE

1. Funding

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2. Potential conflicts of interest

GDS has received grants unrelated to influenza from GSK and Pfizer and travel reimbursement to attend an ad hoc advisory board meeting of GSK also unrelated to influenza; he has provided paid expert testimony in a grievance against a vaccinate-or-mask healthcare worker influenza vaccination policy for the Ontario Nurse Association. JG has received research grants from GlaxoSmithKline Inc. and Hoffman-La Roche Ltd to study antiviral resistance in influenza, and from Pfizer Inc. to conduct microbiological surveillance of *Streptococcus pneumoniae.* MK has received research grants from Roche, Merck, Siemens, Hologic, and Boerhinger Ingelheim for unrelated studies. SS was funded by the Canadian Institutes of Health Research (grant number TPA-90193) and by the Public Health Agency of Canada. Other authors have no conflicts of interest to declare.

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4. Previous presentation of material

Information contained in this manuscript has previously been presented at the Options IX For the Control of Influenza conference on 25 August 2016, Chicago, Illinois, United States and has been accepted for oral presentation at the upcoming 12th Canadian Immunization Conference 6-8 December 2016, Ottawa, Ontario, Canada.

5. Corresponding author information

Danuta M Skowronski MD, FRCPC

BC Centre for Disease Control

655 West 12th Avenue

Vancouver, British Columbia

Canada V5Z 4R4

Ph: 604-707-2511

E-mail: Danuta.Skowronski@bccdc.ca

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F. REFERENCES

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Figure Legend

Figure 1. Epidemic curve of influenza detections by year and influenza type/subtype among Canadian Sentinel Practitioner Surveillance Network (SPSN) patients aged ≥9 years, 2010-11 to 2014-15

Figure 2. Prior vaccination (current and prior season) by year and case status among Canadian SPSN patients aged ≥9 years, 2010-11 to 2014-15

Figure 3. Crude and adjusted vaccine effectiveness (VE) estimates against influenza A(H3N2) among Canadian SPSN patients aged ≥ 9 years, for current season's vaccine (*v2*) regardless of prior season's (±*v1*, ±*v0*) vaccination status, 2010-2011, 2012-13, 2014-15 seasons[a]

VE=vaccine effectiveness; CI= confidence interval; *v2*=current season's vaccine; *v1*=prior season's vaccine; *v0*=vaccine of two prior season's ago

[a] Excluding 2011-12 and 2013-14 season due to small number of A(H3N2) cases.

[b] VE relative to participants not vaccinated in the current season (ø*v2*, ±*v1*, ±*v0*) derived as (1-odds ratio) X 100%

[c] Analyses adjusted for age group, sex, comorbidity, province, collection interval, and week of specimen collection (cubic B-spline functions with 3 equal knots).

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Figure 4. Adjusted vaccine effectiveness (VE) estimates against influenza A(H3N2) by current (*v2*) and/or prior (*v1*) season's vaccination history among Canadian SPSN patients aged ≥9 years, specified by vaccine-virus relatedness conditions and season (2010-11, 2012-13, 2014-15)[a]

VE=vaccine effectiveness; CI= confidence interval

v1=prior season's vaccine; *v2*=current season's vaccine; *e* = epidemic strain

≈ means antigenically related; ≠ means not antigenically related; ≡ means identical; AD = antigenic distance; *v1* - *v2* = comparison between *v1* and *v2*; *v1* - *e* = comparison between *v1* and *e*; *v2* - *e* = comparison between *v2* and *e*

[a] Excluding 2011-12 and 2013-14 season due to small number of A(H3N2) cases.

[b] Analyses adjusted for age group, sex, comorbidity, province, collection interval, and week of specimen collection (cubic B-spline functions with 3 equal knots).

[c] VE relative to participants neither vaccinated in the current nor prior season without taking into account vaccination status two prior seasons ago (*øv2*, *øv1*, ± *v0*)

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Table 1. Summary of influenza A(H3N2) vaccine components and circulating viruses 2010-11, 2012-13 and 2014-15 seasons

Season	2010-11 [8, 19]	2012-13 [9, 19]	2014-15 [10, 19]
***v0* (two prior seasons' vaccine component)**			
WHO-recommended	A/Brisbane/10/2007-like	A/Perth/16/2009-like	A/Victoria/361/2011-like
Egg-adapted HGR	A/Uruguay/716/2007 X-175C	A/Victoria/210/2009 X-187 (clade 1)	A/Victoria/361/2011 IVR-165 (clade 3C)
***v1* (prior season's vaccine component)**			
WHO-recommended	Unchanged from *v0*	Unchanged from *v0*	A/Texas/50/2012-like
Egg-adapted HGR	Unchanged from *v0*	Unchanged from *v0*	A/Texas/50/2012 X-223A (clade 3C.1)
***v2* (current season's vaccine component)**			
WHO-recommended	A/Perth/16/2009-like	A/Victoria/361/2011-like	Unchanged from *v1*
Egg-adapted HGR	A/Victoria/210/2009 X-187 (clade 1)	IVR-165 (clade 3C)	Unchanged from *v1*
***e* (epidemic) A(H3N2) viruses**			
Dominant SPSN clade	Clade 5 (87% of sequenced A(H3N2) viruses)	Clade 3C (94% of sequenced A(H3N2) viruses)	Clade 3C.2a (89% of sequenced A(H3N2) viruses
Number of total and notable[a] hemagglutinin antigenic site amino acid differences between HGR and dominant SPSN clade: notable substitutions displayed by [antigenic site]			
v0 and *v1*	0	0	6, 3: **Q156H [B]***[b]; N226I (RBS) [D]*; T128N (-CHO) [B]
v1 and *v2*	13, 5: **K158N [B]**; **N189K [B];** S138A (RBS) [A]; P194L (RBS) [B]*[c]; S228T (RBS) [D]*	11, 2: **H156Q [B]***; T228S (RBS) [D]*[b]	0
v1 and *e*	11-12, 4: **K158N [B]**; **N189K [B];** S138A (RBS) [A]; P194L (RBS) [B]*	12-14, 3: **N145S [A];** T228S (RBS) [D]*[c]; T128A (-CHO) [B]	10, 5: **N145S [A]**; **F159Y [B]**[b,c]; N226I (RBS) [D]*[d]; N128T (+CHO) [B]; K160T (+CHO) [B]
v2 and *e*	12-13, 1: T228S (RBS) [D]*[c]	5-7, 3: **N145S [A]; Q156H [B]***[b,c]; T128A (-CHO) [B]	10, 5: **N145S [A]**; **F159Y [B]**[b,c]; N226I (RBS) [D]*[d]; N128T (+CHO) [B]; K160T (+CHO) [B]
Antigenic distance (AD) between reference strains[e,f]			
v0 and *v1*	0	0	1
v1 and *v2*	4	1	0
v1 and *e*	7	3	4
v2 and *e*	6	4	4
Summary characterization of vaccine-virus relatedness			
v0 and *v1*	Homologous (*v0* ≡ *v1*)	Homologous (*v0* ≡ *v1*)	Related genetic variant (*v0* ≈ *v1*)
v1 and *v2*	Distinct genetic variant (*v1* ≠ *v2*)	Related genetic variant (*v1* ≈ *v2*)	Homologous (*v1* ≡ *v2*)
v1 and *e*	Distinct genetic variant (*v1* ≠ *e*)	Distinct genetic variant (*v1* ≠ *e*)	Distinct genetic variant (*v1* ≠ *e*)
v2 and *e*	Distinct genetic variant (*v2* ≠ *e*)	Distinct genetic variant (*v2* ≠ *e*)	Distinct genetic variant (*v2* ≠ *e*)

HGR=high growth reassortant; SPSN=Canadian Sentinel Practitioner Surveillance Network; RBS=receptor binding site; +CHO/-CHO=potential gain/loss of glycosylation; AD=antigenic distance

[a] Notable antigenic site amino acid substitutions are those involving a major cluster-transition position in site A or B (bolded), and/or associated with the RBS, and/or with significant potential gain/loss of glycosylation. Asterisks (*) indicate mutations in the egg-adapted HGR itself.
[b] An additional antigenic site D mutation (position 219) in the egg-adapted HGR of *v1* and/or *v2* not displayed.
[c] An additional antigenic site B mutation (position 186) in the egg-adapted HGR of *v1* and/or *v2* not displayed.
[d] An additional non-antigenic site mutation (position 225) of *e* may also be noteworthy for its association with the RBS.
[e] Details provided in Supplement 3 based on reference viruses and antigenic characterizations available in [19].
[f] Antigenic distances derived as $\log_2$fold-difference between homologous and heterologous hemagglutination inhibition (HI) antibody titres for comparator reference viruses, where the first specified virus is the homologous strain (i.e. for *v1* and *v2* comparison, the homologous titre is to *v1*). AD averaged across HI assay repeats for reference strains as specified in Supplement 3. AD of ≥3 corresponds to a ≥8-fold titre difference generally interpreted to signify antigenic distinction between comparator strains. Precise AD values are presented as derived based on reference strains displayed in Supplement 3B, but variability in HI characterization data and therefore derived ADs is acknowledged, as also annotated in footnotes of Supplement 3B.

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Table 2. Participant profile by influenza A(H3N2) case and prior vaccination status among Canadian SPSN patients aged ≥9 years, combined seasons (2010-11, 2012-13, 2014-15)[a,b]

	By case status, n (column %)			By current (v2) and one season's prior (v1) vaccination n (column %)				
	Negative controls	Influenza A(H3N2) cases	p-value	Neither current nor prior	Prior, not current	Current, not prior	Current and prior	p-value
N	**2374**	**1103**		**2003**	**427**	**142**	**905**	
Age group (years)			0.10					<0.01
9-19	316 (13)	166 (15)		346 (17)	61 (14)	21 (15)	54 (6)	
20-49	1246 (52)	563 (51)		1178 (59)	244 (57)	75 (53)	312 (34)	
50-64	551 (23)	231 (21)		400 (20)	93 (22)	33 (23)	256 (28)	
≥65	261 (11)	143 (13)		79 (4)	29 (7)	13 (9)	283 (31)	
Median (range)	40 (9-105)	40 (9-103)	0.60	35 (9-105)	39 (9-92)	39 (9-93)	55 (9-103)	<0.01
Female sex	1500 (63)	637 (58)	<0.01	1187 (59)	266 (62)	92 (65)	592 (65)	0.01
Comorbidity	550 (23)	258 (23)	0.88	299 (15)	106 (25)	29 (20)	374 (41)	<0.01
Province			<0.01					<0.01
Alberta	764 (32)	249 (23)		522 (26)	138 (32)	48 (34)	305 (34)	
British Columbia	465 (20)	158 (14)		400 (20)	67 (16)	26 (18)	130 (14)	
Ontario	756 (32)	449 (41)		636 (32)	162 (38)	48 (34)	359 (40)	
Quebec	389 (16)	247 (22)		445 (22)	60 (14)	20 (14)	111 (12)	
Interval from ILI onset to specimen collection			<0.01					<0.01
0-4 days	1693 (71)	922 (84)		1535 (77)	325 (76)	88 (62)	667 (74)	
5-7 days	681 (29)	181 (16)		468 (23)	102 (24)	54 (38)	238 (26)	
Median (range)	3 (0-7)	3 (0-7)	<0.01	3 (0-7)	3 (0-7)	4 (0-7)	3 (0-7)	<0.01
Month of enrolment			<0.01					<0.01
November	261 (11)	48 (4)		205 (10)	61 (14)	10 (7)	33 (4)	
December	354 (15)	397 (36)		420 (21)	110 (26)	21 (15)	200 (22)	
January	666 (28)	451 (41)		641 (32)	123 (29)	44 (31)	309 (34)	
February	482 (20)	160 (15)		361 (18)	65 (15)	34 (24)	182 (20)	
March	394 (17)	38 (3)		242 (12)	47 (11)	28 (20)	115 (13)	
April	217 (9)	9 (1)		134 (7)	21 (5)	5 (4)	66 (7)	
A(H3N2) status								<0.01
Control	--	--		1364 (68)	276 (65)	119 (84)	615 (68)	
Case	--	--		639 (32)	151 (35)	23 (16)	290 (32)	
Current vaccination (v2)								
Any	799/2439 (33)	330/1120 (29)	0.05	--	--	--	--	
≥2 weeks before ILI onset	734 (31)	313 (28)	0.13	--	--	--	--	
Prior vaccination (v1)			<0.01					
Neither current nor prior	1364 (57)	639 (58)		--	--	--	--	
Prior, not current	276 (12)	151 (14)		--	--	--	--	
Current, not prior	119 (5)	23 (2)		--	--	--	--	
Current and prior	615 (26)	290 (26)		--	--	--	--	

[a] Excluding 2011-12 and 2013-14 seasons due to small number of A(H3N2) cases.
[b] Season-specific information provided in Supplement 4.

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Figure 1

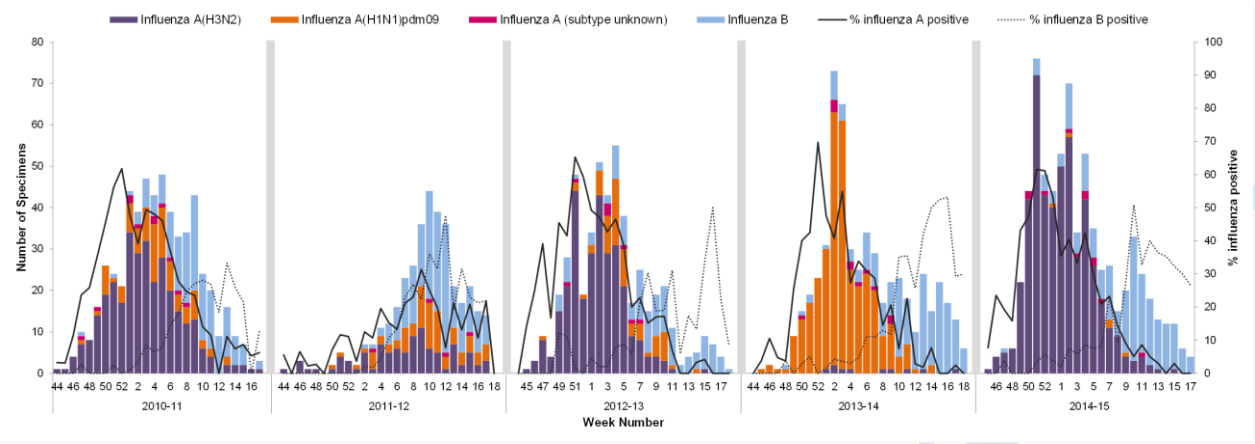

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Figure 2

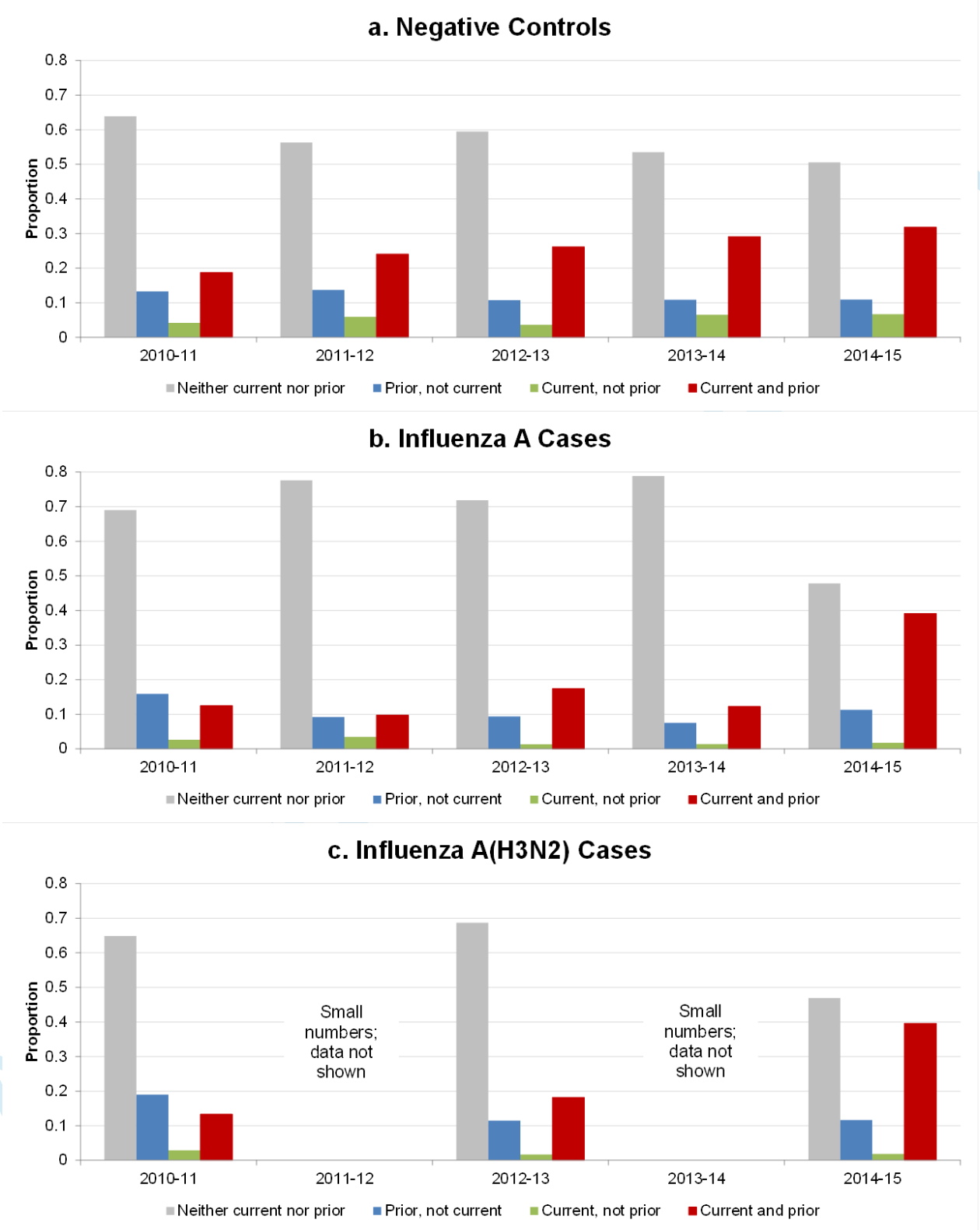
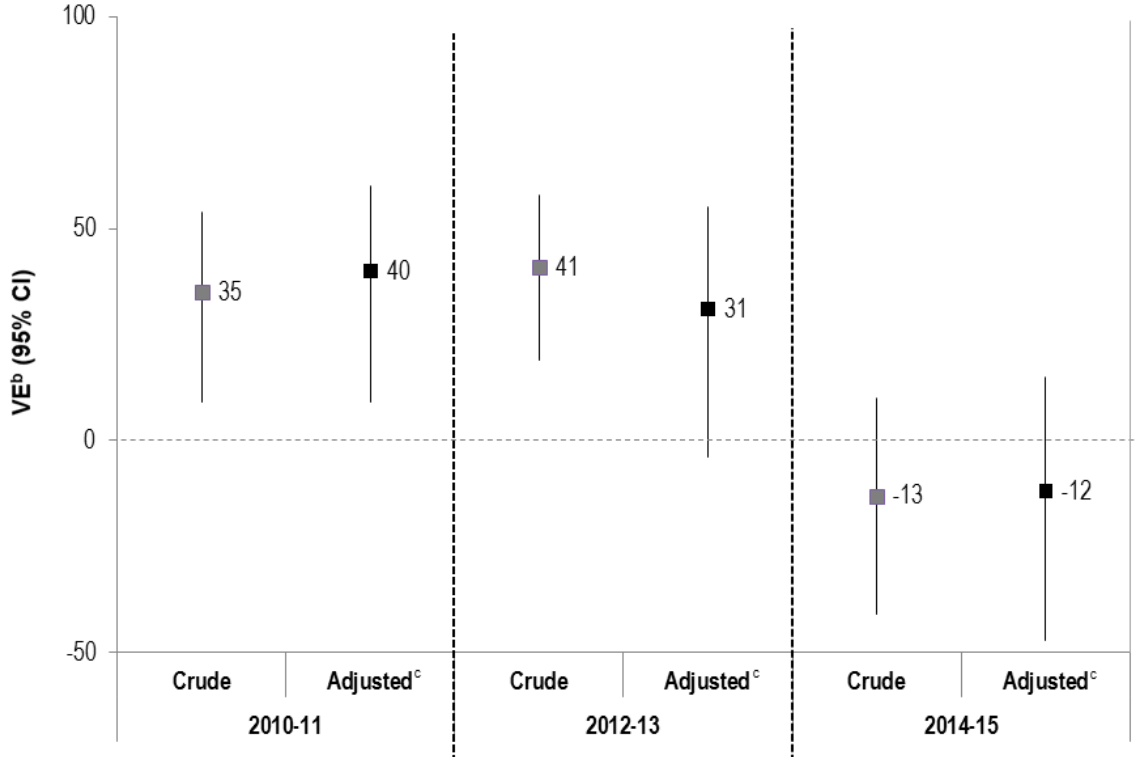

a. Negative Controls
Proportion
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2010-11
2011-12
2012-13
2013-14
2014-15
Neither current nor prior
Prior, not current
Current, not prior
Current and prior
b. Influenza A Cases
Proportion
2010-11
2011-12
2012-13
2013-14
2014-15
Neither current nor prior
Prior, not current
Current, not prior
Current and prior
c. Influenza A(H3N2) Cases
Proportion
Small numbers; data not shown
Small numbers; data not shown
2010-11
2011-12
2012-13
2013-14
2014-15
Neither current nor prior
Prior, not current
Current, not prior
Current and prior

Figure 3



	Cases (n)	Controls (n)	Cases (n)	Controls (n)	Cases (n)	Controls (n)
Not vaccinated (ø*v2*)	265	606	238	465	287	569
Vaccinated (+*v2*)	51	180	59	197	203	357

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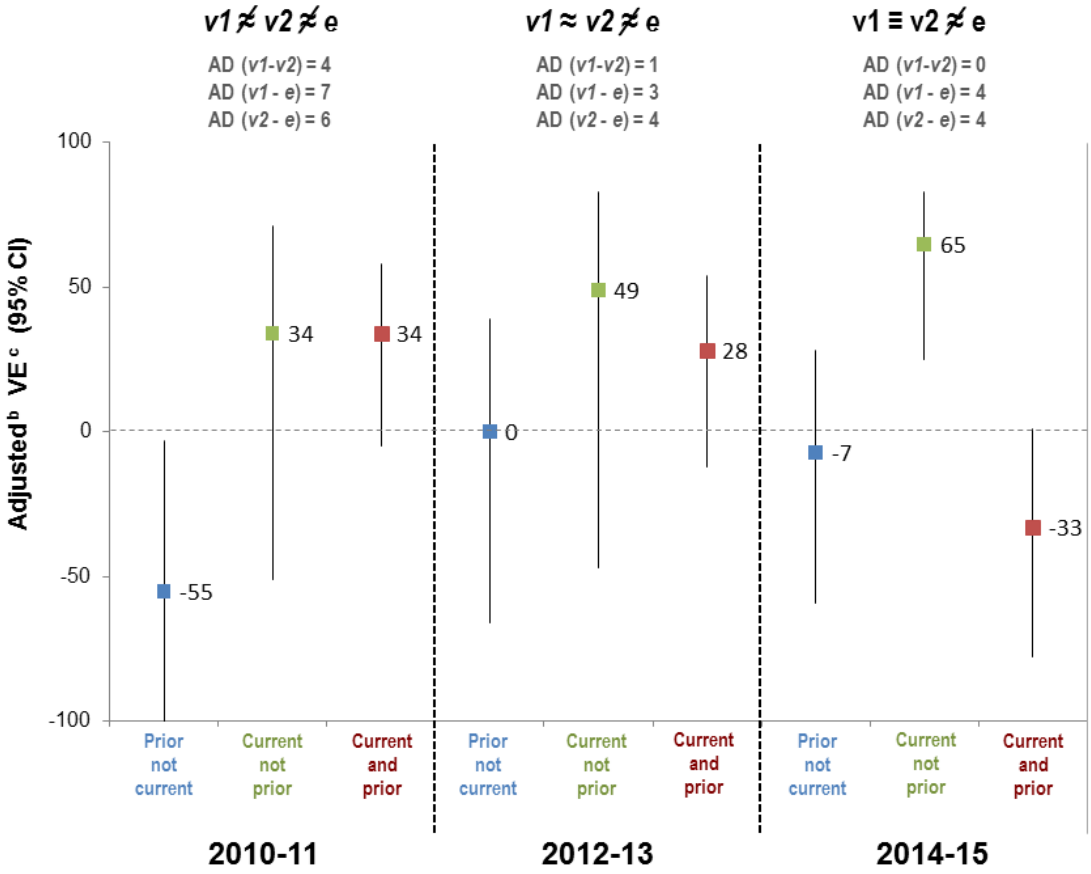
Figure 4



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