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Evaluation of test-negative design estimates of influenza vaccine effectiveness in the context of multiple, co-circulating, vaccine preventable respiratory viruses

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Abstract

Test-negative design (TND) studies are cornerstones of vaccine effectiveness (VE) monitoring for influenza. The introduction of SARS-CoV-2 and RSV vaccines complicate the analysis of this design, with control selection restriction based on other pathogen diagnosis proposed as a solution. We conducted a simulation study and secondary analysis of 2017–18 and 2018–19 TND estimates from a Southeast Michigan ambulatory population to evaluate RSV-status-based control restriction. Simulations suggest that with vaccine-preventable RSV, influenza VE could be moderately biased with RSV prevalence $\geq 25\%$ of controls. Real-world analysis showed 151 influenza-negative adults (10.4 %) had RSV detected from the enrollment nasal swab. There were minimal differences in results of adjusted models with or without RSV exclusion from control groups. Findings suggest that inclusion of RSV cases in the control group of TND studies for influenza VE, particularly where RSV is not vaccine preventable, does not currently pose a major concern for bias in VE estimates.

Keywords

Test-negative design; Bias; Vaccine efficacy

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CRediT authorship contribution statement

Aleda M. Leis: Writing – original draft, Validation, Formal analysis, Data curation, Conceptualization. **Abram Wagner:** Writing – review & editing, Writing – original draft. **Brendan Flannery:** Writing – review & editing, Conceptualization. **Jessie R. Chung:** Writing – review & editing, Conceptualization. **Arnold S. Monto:** Writing – review & editing, Resources, Funding acquisition. **Emily T. Martin:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

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1. Introduction

Before the COVID-19 pandemic, infection with influenza virus resulted in 9–41 million cases of illness, 140,000–710,000 hospitalizations, and 12,000–52,000 deaths annually in the US [1]. Clinical trials for an influenza vaccine began in the 1930s [2], but the Advisory Committee on Immunization Practices (ACIP) did not recommend that everyone ≥ 6 months be vaccinated until the 2010–11 season [3]. The influenza vaccine formulation and effectiveness changes yearly [2], and observational studies conducted seasonally to determine vaccine effectiveness (VE) directly inform recommendations; for example, the ACIP did not recommend a live-attenuated influenza vaccine for the 2016–17 season [4] in the U.S. as a result of earlier VE monitoring showing low effectiveness [4,5].

A common observational method to study VE is the test-negative design (TND), where biospecimens are collected from patients with acute respiratory illness (ARI) presenting to a clinic with either utilization of clinically ordered test results or use of a standardized case definition to prompt prospective specimen collection. The TND was originally designed to reduce confounding from healthcare-seeking behavior, as individuals present for care with a pre-specified set of potential symptoms [6]. Those testing positive for influenza virus are considered cases and those testing negative controls. Logistic regression models are commonly used to compute odds ratios for the association between vaccination and positive test result, which can be converted to a VE estimate [7,8].

A methodological concern in using the TND is the choice of control group, as there are some instances where the control exchangeability assumption may be violated potentially introducing bias. The introduction of COVID-19 and respiratory syncytial virus (RSV) vaccines have raised potential issues of control selection when multiple diseases are vaccine preventable. Already, the TND has been used to estimate COVID-19 VE [9]. Testing positive for these vaccine-preventable viral illnesses could indicate lower probability of any vaccination, and could also indicate changes in the probability of contracting influenza [10]. Additionally, confounding is likely present between receipt of multiple ARI vaccinations [10].

Recent simulation studies have shown that including other vaccine-preventable ARI's, particularly COVID-19, in the control group of influenza VE calculations may underestimate VE [10]. The objective of this paper was to examine the impact and potential biases associated with inclusion or exclusion of those with RSV from the control group when computing VE in an ambulatory population. Given the very recent introduction of the RSV vaccine, we simulate the potential bias introduced to influenza VE estimates assuming RSV was a vaccine preventable infection. We then use the 2017–18 and 2018–19 seasons within the Michigan Henry Ford Influenza Vaccine Effectiveness (MFIVE) TND cohort to estimate effects prior to RSV vaccination introduction, purposefully removing the impact of SARS-CoV-2.

2. Methods

Participants for non-simulation analysis were enrolled in MFIVE in the 2017–18 and 2018–19 influenza seasons. Inclusion criteria, viral testing, and data collection procedures have been previously described [7,11]. Briefly, patients aged ≥ 6 months presenting to an ambulatory care center for ARI who had not received any influenza antiviral treatment for their current illness were eligible for inclusion and collection of a study nasal-throat swab [7,11]. For this analysis, those who received an influenza vaccine <14 days prior to infection, were missing self-reported health data or vaccination status, had an RSV/influenza co-infection, or had their specimen collected >7 days after illness onset were excluded. Laboratory confirmation of infection was determined by RT-PCR for influenza type and subtype using primers and probes provided by the U.S. Centers for Disease Control and Prevention. RT-PCR for RSV infection used a previously reported assay [12]. This study was approved by the Institutional Review Board at our institution (HUM00119183).

2.1. Statistical analysis

Simulation analysis was conducted examining potential bias introduced to influenza VE estimates if RSV was vaccine preventable utilizing the same computational algorithm as Doll et al. [10] with the following parameters: influenza vaccination coverage estimated for a population ≥ 65 years (60 %, 70 %, 80 %) [7,11], RSV vaccination coverage (60 %, 70 %, 80 %), influenza VE (30 %, 40 %, 50 %, 60 %) [13], RSV VE (50 %, 60 %, 70 %), incidence of medically attended influenza (5 %) [14], incidence of medically attended RSV (2.5 %, 5 %) [15], and RSV proportions in the control group (0 %, 10 %, 25 %, 50 %, 75 %, 90 %, 100 %). Risk ratios of 2, 5, and 8, as hypothetical measures of correlation between receipt of both vaccinations, were examined for risk of influenza vaccination given RSV vaccination. A sensitivity analysis examined simulations with no RSV VE. Magnitudes of bias were defined as Doll et al. (moderate bias 10– <20 %, severe bias ≥ 20 %) [10].

Real-world analyses were conducted comparing influenza cases to two control groups: one including those testing positive for RSV (RSV+) and one excluding those RSV+. Due to differences in vaccination patterns across age groups, data were stratified into children (6 months–17 years), adults (18–64 years), and older adults (≥ 65 years). Due to few unvaccinated influenza-positive older adults in the 2018–19 influenza season ($n = 5$), all adults ≥ 18 years were analyzed together in that season. Logistic regression models to compute influenza VE were adjusted for influenza vaccination, age, sex, race, any high-risk condition, self-rated health, and time from symptom onset to specimen collection. Models were run within each age group and control group. Percentage-point differences in VE were computed by subtracting the VE estimate computed excluding RSV+ individuals in the control group from the estimate including RSV+ individuals [10].

3. Results

3.1. Simulations

Simulation analysis (Fig. 1 and Supplemental Table 1) showed little bias for all scenarios when the percentage of RSV+ individuals in the control group was 0 % or 10 %. With

25 % RSV+ in the control group, many scenarios showed moderate bias, particularly with high correlation between receipt of influenza and RSV vaccines. Moderate to significant bias was seen in most scenarios with a majority of controls being RSV+, with magnitude of bias increasing with proportion of RSV in the control group, higher correlation between influenza and RSV vaccination, and larger discordance between influenza and RSV VE. The most extreme bias in influenza VE estimates occurred in simulations of high correlation between receipt of vaccinations with high RSV VE, low influenza VE, and almost all of the control group being RSV+. As expected, there was no bias present in models when RSV VE was negligible (data not shown).

3.2. Real-world analysis

526 adults aged ≥ 18 years were influenza-negative controls for the 2017–2018 influenza season, and 77 were RSV+. 17 were ≥ 65 years of age (18.5 % of controls; Fig. 2) and 60 were 18–64 years of age (13.8 % of controls). There were 74 RSV+ adults for the 2018–19 influenza season (8.0 % of controls). Those in the influenza-positive group, and control groups including and excluding RSV were largely similar (Supplemental Table 2). Those 18–64 in the influenza positive group in 2017–18 and all adults in 2018–19 had higher proportions of specimen collection on days 1–2 after symptom onset than those in control groups. Additionally, those aged 18–64 years in the control group including RSV in the 2017–18 season had an almost 10 % higher rate of influenza vaccination and having any high-risk condition than those influenza positive (58.3 % vs. 48.9 % and 62.2 % vs. 53.2 %, respectively). For the 2017–18 season there was a percentage-point difference in computed model-adjusted VE of -4.5 for those ≥ 65 years of age and 6.3 for those 18–64 years (Table 1). In the 2018–19 influenza season there was a percentage-point difference in computed model-adjusted VE of 1 .

There were 103 RSV+ children included in the control population for the 2017–18 season (25 % of controls; Fig. 2) and 78 RSV+ individuals for the 2018–19 season (17.4 % of controls). Children in the influenza case group for both influenza seasons were significantly older and had higher proportions of specimen collection 1–2 days after symptom onset than those in control groups. Additionally, children in both control groups in the 2017–18 influenza season had almost 20 % higher rates of influenza vaccination than those in the influenza positive group (57.3 % control group excluding RSV+, 59.7 % control group including RSV+, and 34.1 % influenza positive cases). For the 2017–18 season there was a percentage-point difference in computed model-adjusted VE of 0.2 , and in the 2018–19 season there was a percentage-point difference in computed model-adjusted VE of 8.1 (Table 1).

Results of sensitivity analyses excluding those with specimen collection ≥ 5 days after symptom onset and limited to co-circulation of influenza and RSV showed similar results for both children and adults (data not shown); proportions of RSV+ controls in sensitivity analysis were consistent with the full analysis.

4. Discussion

In this simulation study and single-center analysis of influenza VE during the 2017–18 and 2018–19 influenza seasons, we found little evidence that including RSV, another significant ARI, in the control group biased estimates of influenza VE. Simulation analysis showed that when RSV is also a vaccine-preventable illness, influenza VE estimates could be moderately biased with RSV prevalence >10 % of controls, particularly with significant differences in VE between the two vaccinations. Together, these findings suggest that general inclusion of RSV+ in the control group of TND for influenza VE, particularly in populations where RSV is not vaccine-preventable, currently should not significantly bias VE estimates.

Our real-world analysis showed 8–25 % of the control population was RSV+, with this proportion being widely variable by population and season. Controlling for all other viral-positive infections in a TND could yield problems in sample size. A 2016–21 surveillance study of children with acute respiratory illness found that 63 % had some virus detected; notably, 7 % for flu, 17 % for RSV, and 7 % for SARS-CoV-2 [16]. In a study of adult ARI cases from 2015 to 19, 12 % had influenza virus detected and 5 % RSV [17]. Removal of large proportions of the control group may reduce sample size, which could widen confidence intervals, particularly within subgroup analyses, or bias estimates. Additionally, removal of viral positive cases could lead to a sample biased towards chronic diseases with ARI-like symptoms like COPD with a standard case definition [18], which would not be representative of the underlying population from which influenza cases arose.

We found little difference in influenza VE estimates when those RSV+ were included in the control group, compared to VE estimates without RSV+ individuals in the control group. These results are comparable to those by Segaloff et al., who similarly found little difference in VE estimates with varying control populations (anyone negative for influenza, only those positive for other respiratory viruses, and those negative for all common respiratory viruses) in the 2014–15 and 2015–16 influenza seasons [18].

The recent introduction of RSV vaccines for adults ≥ 60 years [19], along with existing programs for COVID-19 and influenza vaccination, creates the potential for up to three vaccine-preventable viral illnesses with similar clinical presentations during ascertainment within the TND. Simulation work by Doll et al. showed the potential for moderate to severe bias in influenza and COVID-19 VE estimates as the proportion of those in the control group with another vaccine-preventable ARI rose, similar to our simulation findings with influenza and RSV [10]. Notably, the simulation models do not account for adjustment of additional confounders such as comorbidities likely to drive the decision to receive one or multiple vaccinations. While our results and that of Doll et al [10] suggest that the currently lower uptake of COVID-19 [8] and RSV [20] vaccines should not independently impact influenza VE estimates, the potential impact of additional RSV-vaccine eligible populations and increasing vaccination coverage towards CDC targets should be considered in analyses of VE estimates.

Strengths of our study include a well-defined population, robust laboratory testing, and replication of methods used in other studies [10]. Given the single-center nature and small

sample size, our VE estimates had wide confidence intervals. Additionally, the current study only considered pre-pandemic influenza and RSV due to data availability; future studies should document the additional impact of SARS-CoV-2 and RSV vaccinations to VE estimation methods in real-world models.

More real-world research is needed to determine the adjusted impact of multiple vaccine-preventable ARI's within the control group of TNDs. This is particularly important where vaccination is not approved for all ages within a cohort, such as with RSV. These findings suggest that general inclusion of RSV in the control group of TND for influenza VE, particularly in populations where RSV remains not vaccine preventable, does not currently pose a major concern for bias in VE estimates against medically-attended influenza illness.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

Data will be made available on request.

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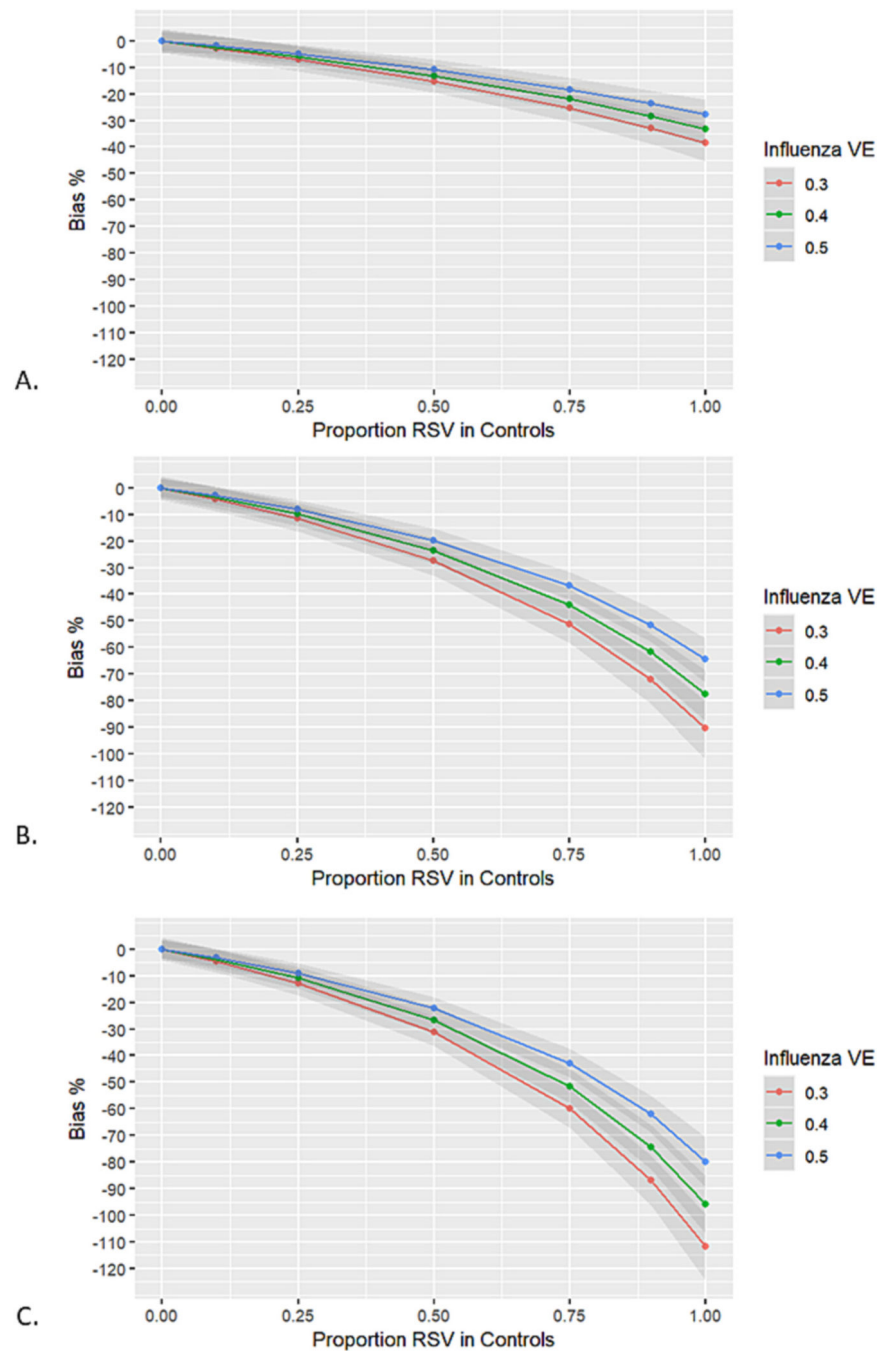
**Fig. 1.**

Illustration showing the results of simulation modeling when the correlation between influenza vaccination and RSV vaccination was a) low ($RR = 2$), b) moderate ($RR = 5$), and c) strong ($RR = 8$). The simulation model included fixed parameters: influenza and RSV vaccination coverage of 60 %, RSV VE of 70 %, and incidence of medically attended influenza or RSV of 5 %. Influenza VE was tested at 30 %, 40 %, and 50 %.

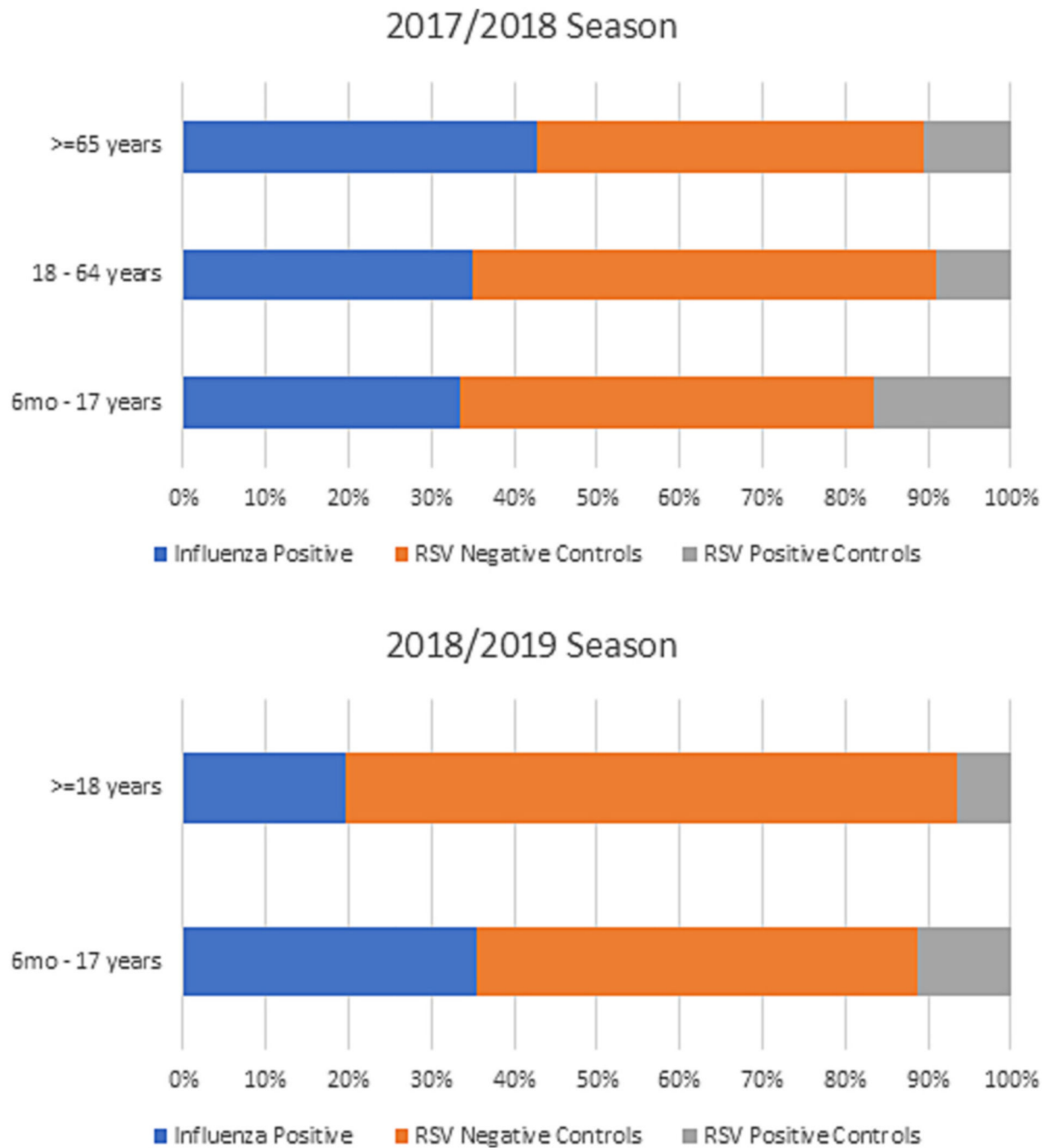


Fig. 2.

Proportions of participants included in the real-world analysis of data who tested positive for influenza, tested positive for respiratory syncytial virus (RSV), or who were controls who tested negative for both influenza and RSV for a) the 2017/2018 influenza season and b) the 2018/2019 influenza season.

Table 1

Computed influenza vaccine effectiveness by age and season for control groups excluding and including individuals who tested positive for RSV.

	No RSV Control	Yes RSV Control	Percentage-point difference $VE_{RSV} - VE_{noRSV}$
2017/2018 Influenza Season			
<i>Adults, 65+ years</i>			
Crude	24.5 % (-73.7, 67.0)	18.4 % (-77.6, 62.5)	-6.1
Fully Adjusted	24.4 % (-89.3, 69.8)	19.9 % (-86.5, 65.6)	-4.5
<i>Adults, 18–64 years</i>			
Crude	26.0 % (-2.8, 46.7)	31.5 % (5.6, 50.2)	5.5
Fully Adjusted	20.3 % (-14.9, 44.8)	26.6 % (-4.8, 48.6)	6.3
<i>Children, 6 months – 17 years</i>			
Crude	61.3 % (44.3, 73.2)	65.0 % (50.5, 75.3)	3.7
Fully Adjusted	55.1 % (33.4, 69.7)	55.3 % (34.8, 69.4)	0.2
2018/2019 Influenza Season			
<i>Adults, 18+ Years</i>			
Crude	10.1 % (-21.7, 33.6)	12.3 % (-18.4, 35.1)	2.2
Fully Adjusted	10.6 % (-25.3, 36.3)	11.6 % (-23.6, 36.7)	1.0
<i>Children, 6 months – 17 years</i>			
Crude	15.0 % (-17.8, 38.7)	26.1 % (-1.4, 46.1)	11.1
Fully Adjusted	5.6 % (-35.9, 34.4)	13.7 % (-23.3, 39.6)	8.1

Models were adjusted for age, sex, race, any high-risk condition, self-rated health, and time from symptom onset to specimen collection.