Homologous and heterologous immunity comparison between Fluzone High-Dose and Standard-Dose influenza vaccine: a human vaccine cohort study from 2014 to 2018

Authors

Yang Ge^, W. Zane Billings, Wannan Cao, Amanda L. Skarlupka, Ye Shen, Justin Bahl, Paul Thomas, Ted M. Ross, Andreas Handel^,

Authors affiliations

: School of Health Professions, University of Southern Mississippi, Hattiesburg, 39402, MS, USA; <https://orcid.org/0000-0001-5100-0703>; : College of Public Health, The University of Georgia, Athens, 30606, GA, USA; : Center for the Ecology of Infectious Diseases, The University of Georgia, Athens, 30606, GA, USA. : School of Public Health, Peking University, Beijing, China; : National Cancer Institute, Bethesda, 20814, MD, USA; : St. Jude Children’s Research Hospital, Memphis, 38105, TN, USA; : Center for Vaccines and Immunology, The University of Georgia, Athens, 30606, GA, USA; : Cleveland Clinic, Port St. Lucie, 34952, FL, USA;

^ Corresponding authors: [yang.ge@usm.edu](mailto:yang.ge@usm.edu), [ahandel@uga.edu](mailto:ahandel@uga.edu)

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# Abstract

**Background**: The high-dose (HD) Fluzone influenza vaccine is provided to the elderly population due to the limited effectiveness of the standard-dose (SD) version. While HD may enhance protection against the same vaccine strain (homologous protection), research on its impact regarding protection against different strains (heterologous protection) is limited. We aim to compare the antibody immune responses for both homologous and heterologous protection between SD and HD vaccines using human vaccine cohort study

**Methods**: We extracted data from a human vaccine cohort study involving volunteers who received either the SD or HD Fluzone vaccine during five flu seasons from 2014 to 2018. After transforming the data into log2 titer units, we employed a Bayesian multilevel modeling framework to investigate how the vaccine dosage affects the levels of serological hemagglutination inhibition titer (HAI) for both homologous and heterologous immunogenicity.

**Results**: During these five flu seasons, HD and SD vaccine were given 198 and 160 times, respectively. Despite updates to vaccine strains during these seasons, our analysis revealed that the HD vaccine generally exhibited slightly better homologous immunogenicity compared to the SD vaccine. Specifically, across all seasons, the HD vaccine showed an overall increase in homologous HAI titer by 0.26 (95%CrIs, -0.42 to 0.92) (CrIs: equal-tailed credible interval). However, the superiority of the HD vaccine was not consistent when it came to heterologous immunogenicity. For instance, in the case of the H1N1-California-2009 strain, the HD vaccine demonstrated lower heterologous immunogenicity than the SD vaccine, with a titer increase difference (HD - SD) of -0.08 (95%CrIs, -0.18 to 0.03). Furthermore, we observed that the HD vaccine had lower titer increases compared to the SD vaccine in the 2016/17 (-0.32 (95%CrIs, -0.45 to -0.18)) and 2018/19 (-0.2 (95%CrIs, -0.5 to 0.08)) flu seasons. These findings remained similar across various outcomes (seroconversion and seroprotection) and different analytical methods (non-multilevel models).

**Conclusions**: The HD influenza vaccine consistently enhances homologous protection but varies significantly in its effectiveness against different strains. When there’s a significant vaccine-strain mismatch in a season, HD might not be the best option. Further dose optimization studies are required.

# Introduction

Influenza vaccines are widely used to protect humans against influenza infections, but the average effectiveness is only around 50% [1]. The licensed standard-dose (SD) split-inactivated virion vaccine, containing 15 micrograms of HA antigen for each strain, exhibited low immunogenicity and limited protection, especially in the elderly population [2]. To address this, a high-dose (HD) influenza vaccine, Sanofi Pasteur Fluzone HD, was developed, having 60 micrograms of HA for each vaccine strain [3,4]. This increased dosage improved homologous protection against the vaccine strain, leading to its licensing in the US in 2009 and subsequent administration to individuals aged 65 years and older [4–8].

Maximizing both homologous and heterologous protection is a key goal in developing a universal influenza vaccine, as recognized in previous research [9,10]. Increasing the vaccine dose appears to enhance homologous immunogenicity [5,11], but its impact on heterologous immunogenicity remains uncertain. Notably, several antigens have shown non-monotone patterns in dose-dependent immune responses [12–15], indicating that vaccine dosage can produce diverse effects. For instance, a higher dose might trigger a broader B-cell or T-cell response, simultaneously enhancing both strength and breadth of protection. However, it could also selectively stimulate B-cell or T-cell responses only to specific strains, resulting in strong yet narrower protection. Therefore ,the potential competition among HD vaccine-induced B-cells or T-cells might narrow vaccine protection [16], making high-risk populations more vulnerable to vaccine mismatches. Thus, the true impact of the HD influenza vaccine on heterologous protection remains a critical question that has yet to be thoroughly explored.

The choice of vaccine dose plays a crucial role in efficacy, side effects, costs, and availability [5,15]. To have a dose optimized vaccine, it’s essential to thoroughly assess the impact of dosage. In this study, we compared both homologous and heterologous vaccine immunogenicity between HD and SD vaccines, using data from a human vaccine cohort study in which volunteers received either vaccine type across five flu seasons.

# Methods

**Data source**. We extracted human vaccination data between the 2014/15 to the 2018/19 flu seasons from our ongoing vaccination cohort study. In this study, investigators annually recruited individuals who hadn’t received an influenza vaccine for the season prior to the flu season’s onset. Individuals aged 65 and older were given the choice between the HD and SD vaccines. The HD vaccine was trivalent, while the SD vaccines were trivalent in 2014 and quadrivalent in subsequent years, with the quadrivalent formulation containing an additional B-strain. Investigators collected serological samples both before vaccination and 21-28 days after vaccination. For a detailed description of the study and data collection methodology, please refer to previous publications [17–19].

**Data processing**. The amount of hemagglutination-inhibition (HAI) antibody in those serological samples were determinted by HAI standard dilution assays. Thus, our raw data include HAI titers before and after vaccination with different testing strains. The assay’s limit of detection (LOD) was 1:10, and values below the LOD were recorded as 1:5 in the raw data. Following previous studies [20,21], we transformed HAI titer measurements using the formula . This transformation placed HAI values on a scale from zero (LOD) to 12 (the highest reported dilution of 1:20480). As the trivalent HD vaccine, differ from the SD, only contained a single B strain. To study the impact of influenza A subtypes strains, we excluded results related to all B strains in this study.

**Outcome definitions**. We calculated four common outcomes used in influenza vaccine studies [4,20]. The first two outcomes are on an integer scale (after transformation of titer as explained earlier): 1) titer increase, which is the difference between post-vaccination and pre-vaccination titers, and 2) post-vaccination titer. The other two outcomes are categorical versions of the first two, namely: 3) seroconversion, defined as either a pre-vaccination titer of 0 (equal to the LOD) with a post-vaccination titer of at least 3, or a pre-vaccination titer above the LOD with a post-vaccination titer at least 2 units higher (equivalent to a dilution measurement of < 1:10 pre-vaccination and >= 1:40 post-vaccination or a >=4-fold increase from pre- to post-vaccination in dilution units) [4]. Finally, 4) seroprotection is defined as a post-vaccination HAI titer of 3 or greater (equal to or greater than a 1:40 dilution in the original units), which is widely considered a threshold for protection [22].

**Statistical analyses**. In the descriptive analyses, we report mean and standard deviation for the continuous outcomes of titer increase and post-vaccination titer, and counts and percentages for the binary outcomes, including seroconversion and seroprotection. To estimate the impact of vaccine dose while considering study multilevel features (Supplementary material), we employed a Bayesian multilevel modeling approach. Our models also included age and pre-vaccination titer as covariates. Finally, we estimated the strain-specific and vaccine-specific effects of vaccine dose using multilevel models [23]. Notably, the inclusion of pre-vaccination titer as a covariate led to mathematically identical dose coefficient estimations for titer increase and post-vaccination titer outcomes, and therefore, we did not fit multivariable models for the post-vaccination titer outcome.

We summarized the impact of the HD vaccine relative to the SD vaccine using the Highest Maximum A Posteriori probability estimate (MAP) and a 95% Equal-Tailed Credible Interval (CrIs) [24,25]. To assess the robustness of our multilevel models, we compared them to generalized linear models (GLM), and the results are available in the supplementary material.

**Implementation**. All analyses were completed using R [26]. The package of brms [27] was used for the Bayesian multilevel analysis. Detailed descriptions of all models and the code for running the analyses can be found in the supplementary material.

# Results

## Data Description

Our samples cover the influenza seasons from 2014 to 2018. During this period, the HD and SD vaccines were given 198 and 160 time, respectively to individuals aged 65 years and older (Table 1). Throughout the study’s duration, two H1N1 vaccine strains (H1N1-California-2009 in the 2014/15 and 2015/16 seasons and H1N1-Michigan-2015 in the 2017/18 and 2018/19 seasons) and four H3N2 strains (H3N2-Texas-2012 in the 2014/15 season, H3N2-Switzerland-2013 in the 2015/16 season, H3N2-Hong Kong-2014 in the 2016/17 and 2017/18 seasons, H3N2-Singapore-2016 in the 2018/19 season) were included in the vaccines.

Table 1: Description of the study and homologous response in High-dose (HD) and Standard-dose (SD) group. Sample size and vaccine seasons are indicated for each vaccine strain.

|  |  |  |
| --- | --- | --- |
| **Variables** | **Standard-dose vaccine** | **High-dose vaccine** |
| **Sample size (N)** | 160 | 198 |
| **Age (median, IQR)** | 68.00 [66.00, 74.00] | 70.00 [68.00, 75.00] |
| **H1N1-California-2009** | N = 90 (2014, 2015, 2016) | N = 94 (2014, 2015, 2016) |
| - Prior-vaccination titer, median [IQR] | 20.00 [5.00, 40.00] | 20.00 [10.00, 40.00] |
| - Post-vaccination titer, median [IQR] | 40.00 [20.00, 80.00] | 80.00 [40.00, 160.00] |
| - Titer increase, mean (SD) | 1.13 (1.11) | 1.53 (1.34) |
| - Seroconversion, n (%) | 29 (22.8) | 69 (39.7) |
| - Seroprotection, n (%) | 76 (59.8) | 138 (79.3) |
| **H1N1-Michigan-2015** | N = 25 (2017, 2018) | N = 19 (2017, 2018) |
| - Prior-vaccination titer, median [IQR] | 20.00 [10.00, 40.00] | 20.00 [10.00, 40.00] |
| - Post-vaccination titer, median [IQR] | 40.00 [40.00, 80.00] | 40.00 [40.00, 100.00] |
| - Titer increase, mean (SD) | 0.85 (1.06) | 1.38 (1.21) |
| - Seroconversion, n (%) | 7 (21.2) | 10 (41.7) |
| - Seroprotection, n (%) | 26 (78.8) | 22 (91.7) |
| **H3N2-Texas-2012** | N = 53 (2014) | N = 39 (2014) |
| - Prior-vaccination titer, median [IQR] | 40.00 [20.00, 160.00] | 40.00 [20.00, 80.00] |
| - Post-vaccination titer, median [IQR] | 160.00 [80.00, 320.00] | 160.00 [80.00, 160.00] |
| - Titer increase, mean (SD) | 1.77 (1.93) | 1.69 (1.66) |
| - Seroconversion, n (%) | 27 (50.9) | 20 (51.3) |
| - Seroprotection, n (%) | 48 (90.6) | 36 (92.3) |
| **H3N2-Switzerland-2013** | N = 40 (2015) | N = 58 (2015) |
| - Prior-vaccination titer, median [IQR] | 40.00 [17.50, 80.00] | 40.00 [20.00, 80.00] |
| - Post-vaccination titer, median [IQR] | 320.00 [80.00, 320.00] | 320.00 [80.00, 640.00] |
| - Titer increase, mean (SD) | 2.92 (1.77) | 2.97 (1.68) |
| - Seroconversion, n (%) | 31 (77.5) | 45 (77.6) |
| - Seroprotection, n (%) | 38 (95.0) | 57 (98.3) |
| **H3N2-Hong Kong-2014** | N = 48 (2016, 2017) | N = 92 (2016, 2017) |
| - Prior-vaccination titer, median [IQR] | 40.00 [20.00, 80.00] | 40.00 [20.00, 80.00] |
| - Post-vaccination titer, median [IQR] | 80.00 [40.00, 320.00] | 160.00 [80.00, 320.00] |
| - Titer increase, mean (SD) | 1.41 (1.73) | 2.13 (1.53) |
| - Seroconversion, n (%) | 19 (33.9) | 53 (57.0) |
| - Seroprotection, n (%) | 51 (91.1) | 86 (92.5) |
| **H3N2-Singapore-2016** | N = 11 (2018) | N = 8 (2018) |
| - Prior-vaccination titer, median [IQR] | 40.00 [15.00, 80.00] | 10.00 [5.00, 40.00] |
| - Post-vaccination titer, median [IQR] | 80.00 [30.00, 80.00] | 30.00 [16.25, 50.00] |
| - Titer increase, mean (SD) | 0.55 (0.69) | 0.75 (0.89) |
| - Seroconversion, n (%) | 1 ( 9.1) | 1 (12.5) |
| - Seroprotection, n (%) | 8 (72.7) | 4 (50.0) |

When comparing HD and SD vaccines for each season, we observed variations in titer increase. Overall, the HD vaccine showed higher titer increases against homologous strains, but lower responses to heterologous strains in the 2016 and 2018 seasons (Supplementary material).

## HD vaccines led to increased strain-specific homologous responses

Following vaccination with the HD vaccine, we observed stronger homologous HAI responses specific to the vaccine strains compared to the SD vaccine (Figure 1). Both H1N1 vaccine strains demonstrated an increased response with HD. The impact of HD on H3N2 vaccine strains showed more variability, with the titer increase outcome for the Texas-2012 vaccine showing a slightly reduced impact of the HD vaccine, although it was very close to the no-effect line.

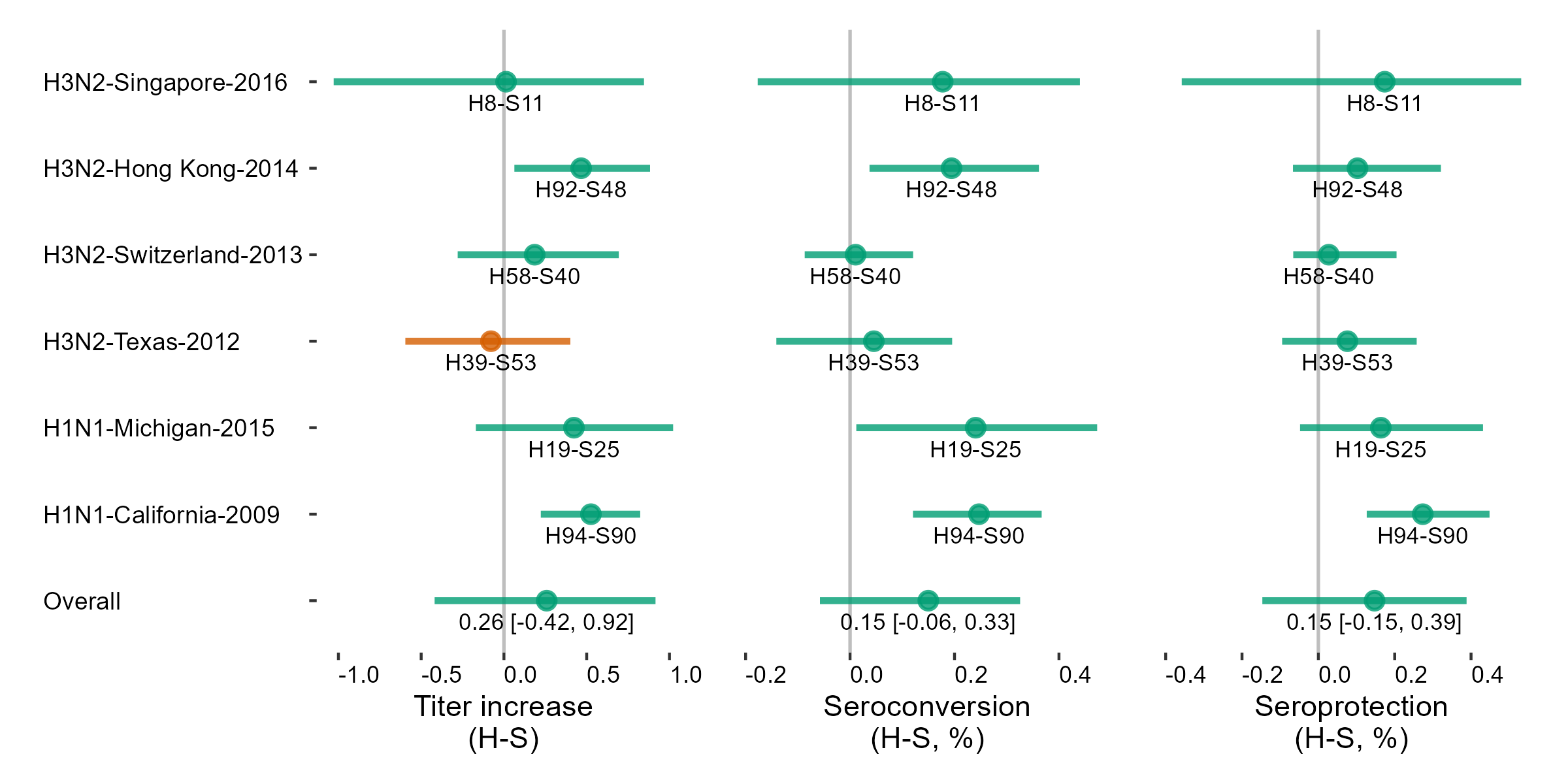


Figure 1: The impact of HD vaccine compared to SD on strain-specific, homologous HAI responses. The MAP and 95% equal-tailed credible interval (CrIs) of the overall effect (HD vs. SD) are shown. The numbers under each line show the sample size (H: HD; S: SD) for that specific strain or the overall effect size.

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