NEJM Evidence

Published March 3, 2026

DOI: 10.1056/EVIDpha2600007

PUBLIC HEALTH ALERTS | IN PARTNERSHIP WITH CIDRAP

Antibodies Elicited by the 2025–2026 Influenza Vaccine

Jiaojiao Liu, Ph.D.,[1] Shuk Hang Li,[1] Naiqing Ye,[1] Tachianna Griffiths,[1] Elizabeth M. Drapeau, Ph.D.,[1] Reilly K. Atkinson,[1] Ronald G. Collman, M.D.,[2] and Scott E. Hensley, Ph.D.[1]

Abstract

A new H3N2 variant (named subclade K) possesses several key hemagglutinin substitutions and is circulating widely during the 2025–2026 influenza season. We sought to determine whether the 2025–2026 seasonal influenza vaccine elicits antibodies in humans that recognize this variant. We found that H3N2 subclade K viruses are antigenically advanced; however, the 2025–2026 seasonal influenza vaccine elicited antibodies in many individuals that efficiently recognized these viruses. Thus, the current seasonal influenza vaccine likely will be partially effective at preventing illness associated with H3N2 subclade K virus infections.

The Public Health Alerts section in *NEJM Evidence* is in partnership with the Center for Infectious Disease Research and Policy (CIDRAP) at the University of Minnesota.

Introduction

Seasonal influenza viruses continuously undergo antigenic drift and vaccine antigens are updated regularly. A new H3N2 variant (named subclade K) emerged in the spring and summer of 2025 and is now circulating widely in the United States and other parts of the world.[1] The hemagglutinin (HA) protein of H3N2 subclade K viruses possesses 11 substitutions (K2N, T135K, S144N, N145S, N158D, I160K, Q173R, A186D, K189R, T328A, HA2: S49N) relative to the HA of the Northern Hemisphere 2025–2026 H3N2 vaccine strain, many of which are in antibody-binding epitopes (Fig. 1A). In September, the World Health Organization reported that ferret antisera raised against the current H3N2 vaccine strain poorly reacted to subclade K H3N2 viruses,[2] raising the concern that there could potentially be a major vaccine mismatch during the 2025–2026 Northern Hemisphere influenza season. H3N2 subclade K viruses circulated widely in England during the fall of 2025, and early-season vaccine effectiveness estimates against influenza-related emergency department visits and hospital admissions were unexpectedly high.[3] It is therefore important to determine whether the 2025–2026 influenza vaccine elicits antibodies in humans that recognize H3N2 subclade K viruses.

Methods

HUMAN SERUM SAMPLES

Serum samples from 76 adults (ages 24–81 years) were collected before and 27–30 days after they received a standard dose of the egg-based 2025–2026 Flulaval Trivalent

The author affiliations are listed at the end of the article.

Scott E. Hensley can be contacted at hensley@pennmedicine.upenn.edu.

influenza vaccine (GlaxoSmithKline) between October and November 2025. Vaccines for this study were purchased using funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under contract no. 75N93021C00015. Participants were recruited from the University of Pennsylvania campus and surrounding communities using flyers and electronic notifications distributed through patient portals. The majority (69 of 76) of participants reported receiving an influenza vaccine during the previous 2024–2025 season. Participants were enrolled using predefined birth-year groups (before 1957, 1957–1967, 1968–1976, 1977–1989, and after 1989) to ensure balanced representation across birth cohorts. We excluded individuals for enrollment if they had a prior severe influenza vaccine reaction, were immunosuppressed or recently received immunosuppressive therapy, recently received blood products or experimental agents, had active or recent malignancies, had prolonged glucocorticoid use, were pregnant, intended to donate blood, or if they had any condition deemed unsafe or likely to interfere with study participation by the investigative team. This study was approved by the Institutional Review Board of the University of Pennsylvania.

VIRUSES

Influenza viruses with HA and neuraminidase of the 2025–2026 H3N2 vaccine strain (A/Croatia/10136RV/2023; GISAID accession numbers EPI_ISL_19296516) and an H3N2 subclade K virus (A/New York/GKISBBBG87773/2025; GISAID accession numbers EPI_ISL_20126669) were prepared as described in the 'Viruses' section in the Supplementary Appendix.

HEMAGGLUTINATION INHIBITION ASSAYS

Hemagglutination inhibition (HAI) assays were performed as previously described.[4] Detailed methods are provided in the 'HAI Assays' section in the Supplementary Appendix.

STATISTICAL ANALYSES

We use descriptive statistics to report HAI titers before and after vaccination and to examine the difference in year of birth between seronegative and seropositive participants. The trend lines of HAI titers by year of birth are locally estimated scatterplot smoothing curves (smoothing parameter=0.7) with 95% confidence intervals. Confidence intervals have not been adjusted for multiplicity and should not be used in place of hypothesis testing.

Results

We collected sera from 76 individuals before and approximately 1 month (between 27 and 30 days) after they received a standard dose of the egg-based 2025–2026 Flulaval Trivalent influenza vaccine. We completed HAI assays to identify antibodies that block virus attachment of the 2025–2026 H3N2 vaccine strain and an H3N2 subclade K variant virus. Antibody titers against both the 2025–2026 H3N2 vaccine strain and H3N2 subclade K virus increased in sera from most individuals following vaccination; however, antibody geometric mean titers were approximately twofold higher to the 2025–2026 H3N2 vaccine strain compared to the H3N2 subclade K virus after vaccination (Fig. 1B and 1C). Before vaccination, 39% (30 of 76) of participants were seropositive (≥40 HAI titer) against the 2025–2026 H3N2 vaccine strain and 11% (8 of 76) of participants were seropositive against the H3N2 subclade K virus. Following vaccination, 71% (54 of 76) and 39% (30 of 76) of participants were seropositive against the 2025–2026 H3N2 vaccine strain and the H3N2 subclade K virus, respectively. We did not observe major apparent birth-year-related differences in antibody reactivity to either virus (Fig. 1D and 1E), and we observed similar birth year distributions among H3N2 subclade K seronegative and seropositive individuals following vaccination (Fig. 1F).

Conclusions

These data suggest that H3N2 subclade K viruses are antigenically advanced compared to the 2025–2026 H3N2 vaccine strain; however, the antigenic differences that we observed in sera from some humans are not as large as previously reported in ferrets.[2] Although we found that human antibodies elicited by vaccination reacted more efficiently to the H3N2 vaccine strain relative to subclade K H3N2 viruses, many individuals produced antibodies that efficiently recognized subclade K H3N2 viruses after vaccination.

We only evaluated antibody responses elicited by standard doses of a conventional egg-based influenza vaccine. Further, we only evaluated HAI antibodies that block viral attachment. Future research should evaluate both cellular and humoral immune responses elicited by other types of vaccine, including high dose vaccines in older adults. Most participants (69/76) in this cohort received an influenza vaccine during the previous 2024–2025 season. Future research should evaluate immune responses elicited by the

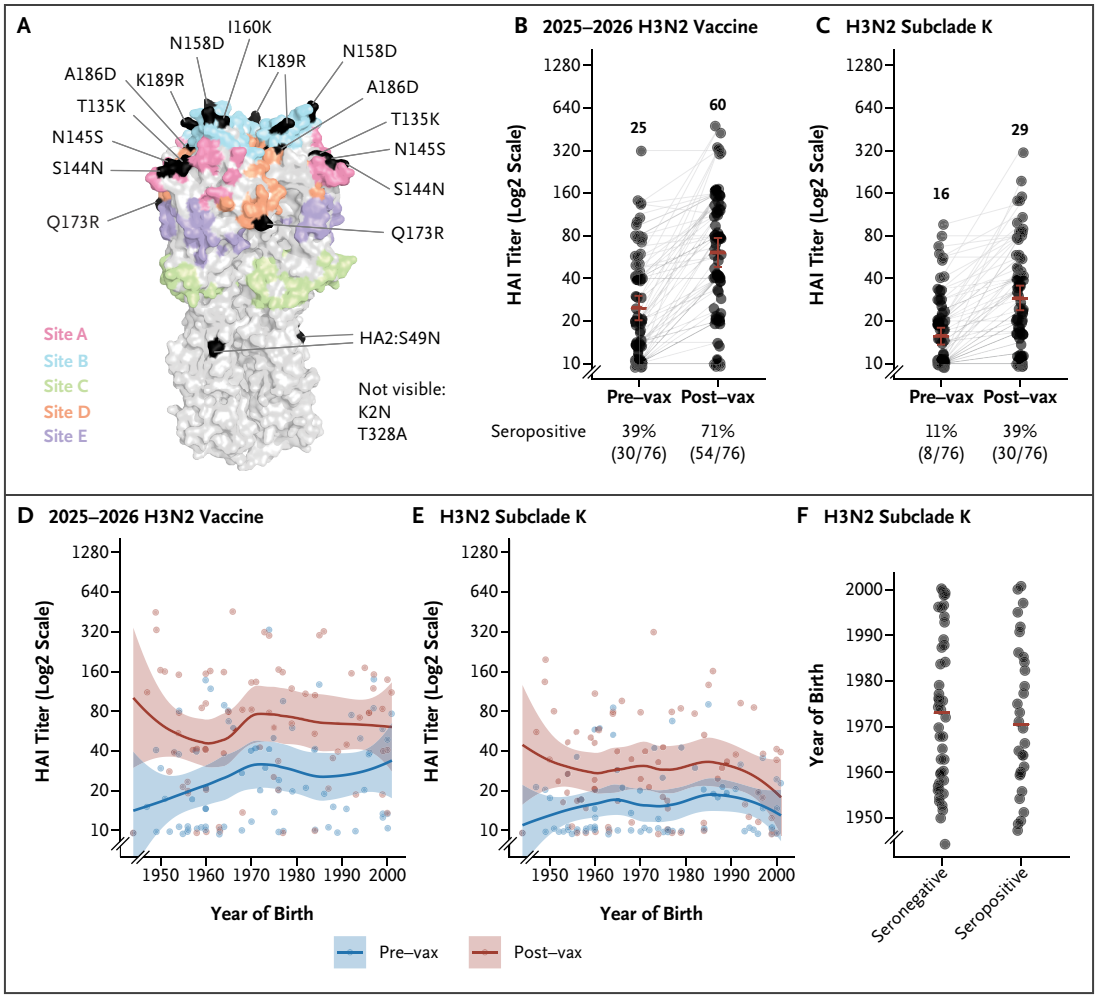

Figure 1. Human Antibody Recognition of H3N2 Subclade K Virus.

Panel A shows the crystal structure of the A/Victoria/22/2020 hemagglutinin (HA) trimer (PDB_8FAQ) with major antigenic sites (Sites A–E) displayed. Amino acid differences between the HA of the 2025–2026 H3N2 vaccine strain (A/Croatia/10136RV/2023) and H3N2 subclade K virus (A/New York/GKISBBBG87773/2025) are shown in black. Panels B and C show hemagglutination inhibition (HAI) titers to the 2025–2026 H3N2 vaccine (vax) strain (A/Croatia/10136RV/2023; a subclade J.2 virus) and an H3N2 subclade K variant virus (A/New York/GKISBBBG87773/2025) using sera collected from 76 adults before and after (27–30 days) they received the 2025–2026 influenza vaccine. Each point in Panels B and C represents the geometric mean titer using serum from one individual tested in two independent experiments. Lines in Panels B and C connect the same individual. Seropositivity was defined as an HAI titer ≥40. HAI titers are plotted as the geometric mean titer with 95% confidence interval. Panels D and E display these data with each participant's birth year indicated on the *x* axes. The trend lines in Panels D and E are locally estimated scatterplot smoothing curves (smoothing parameter=0.7) with 95% confidence intervals. Panel F shows the birth-year distributions among H3N2 subclade K seronegative and seropositive individuals after vaccination. The median year of birth is shown as a red line. Confidence intervals have not been adjusted for multiple comparisons and should not be used in place of hypothesis testing.

2025–2026 influenza vaccine in individuals with different vaccine histories.

Overall, these data suggest that the 2025–2026 influenza vaccine induces antibodies in many vaccine recipients that are considered, from a regulatory perspective, likely to provide protection against H3N2 subclade K viruses.

Disclosures

Author disclosures and other supplementary materials are available at evidence.nejm.org.

The viruses in this manuscript were created using sequences obtained from GISAID. We gratefully acknowledge all data contributors, i.e., the Authors and their Originating laboratories responsible for obtaining the specimens, and their Submitting laboratories for generating the

genetic sequence and metadata and sharing via the GISAID Initiative (Table S1).

Funded in part with federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under contract no. 75N93021C00015 (to Dr. Hensley).

Author Affiliations

[1] Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

[2] Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

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