Clinical Infectious Diseases

MAJOR ARTICLE

Influenza A and B Viral Shedding in Adults Immunized with Live Attenuated Influenza Vaccine

Brandon M. Tower[1], Cindy M. Liu[1], David J. Diemert[2], Sydney G. Nelson[1], Chelsea E. Ware[3], Juan E. Salazar[1], Tony Pham[1], Reed M. Leventis[1], Katherine E. Rose[1], Annie L.S. Roberts[1], Nathan O. Weber[1], Edward H. Sung[1], Maliha Aziz[1], Ryan M. Troyer[4], Daniel E. Park[1*]

[1]Antibiotic Resistance Action Center, Department of Environmental and Occupational Health, Milken Institute School of Public Health, George Washington University, Washington, DC, 20052, USA; [2]George Washington University School of Medicine and Health Sciences, Department of Microbiology, Tropical Medicine and Immunology, Washington, DC, 20052, USA; [3]George Washington Vaccine Research Unit, George Washington Medical Faculty Associates, School of Medicine and Health Sciences, George Washington University, Washington, DC, 20052, USA; [4]Department of Microbiology and Immunology, Schulich School of Medicine & Dentistry, Western University, London, ON, Canada

Background. Live attenuated influenza vaccine (LAIV) induces mucosal immunity through limited viral replication in the upper respiratory tract, but post-vaccination viral shedding dynamics and their clinical correlates remain incompletely characterized.

Methods. We evaluated 283 healthy adults (108 in 2023–2024, 175 during the 2024–2025 influenza seasons) following intranasal LAIV administration. Nasal swabs were collected on post-LAIV days 1, 2–4, and 5–7 to quantify influenza A and B RNA by RT-PCR. Viral detection, shedding duration and burden, clearance kinetics, and probability of detection were compared

CORRESPONDING AUTHOR: Daniel E. Park, Antibiotic Resistance Action Center, Department of Environmental and Occupational Health, Milken Institute School of Public Health, George Washington University, Washington, DC 20052, USA. danpark@gwu.edu

DOI: 10.1093/cid/ciag294

across seasons, vaccine strain compositions (quadrivalent vs. trivalent), and host factors. Respiratory symptoms were assessed.

Results. Early viral shedding was frequent: influenza A or B RNA was detected in 86.9% of participants on day 1, with co-detection in 52.7%. Probability of detection declined from 92% on day 1 to 9% on day 7. Influenza B had longer shedding duration (median, 2.0 vs. 1.0 days; $p<0.001$) and higher shedding burden (4.2 vs. 4.0 $\log_{10}$ RNA copies/mL; $p<0.001$). Three clearance profiles – rapid (clearance ≤4 days), moderate (5–7 days), and slow (≥7 days) – were identified. Profiles were consistent across seasons but influenza B was disproportionately represented in slower-clearance groups ($p<0.001$). Total viral burden ($p<0.01$) and shedding duration ($p=0.01$) were associated with total symptom burden.

Conclusions. LAIV replication begins within 24 hours and declines over the first week, with persistent shedding uncommon after day 7. Influenza B replicates more extensively and persists longer than influenza A. Symptom burden correlates with shedding duration and viral burden.

Keywords. Influenza; live attenuated influenza vaccine; viral shedding; immunization

INTRODUCTION

Vaccination remains the most effective strategy for preventing severe influenza infections,[1] which continue to cause substantial morbidity and mortality worldwide. The live attenuated influenza vaccine (LAIV) was first approved in 2003 in the United States for use in qualified individuals aged 5-49 years.[2] LAIV is administered intranasally and replicates in the upper respiratory tract, mimicking a natural infection, generating humoral and mucosal immune responses.[3,4] Despite these advantages, LAIV efficacy has varied considerably across influenza seasons,[5,6] and is generally considered more immunogenic in pediatric populations.[4,7,8] This highlights the need to better understand host and vaccine factors that shape LAIV outcomes such as post-vaccination viral shedding, which has been linked to the magnitude of immune responses.[9,10]

Most studies of post-LAIV shedding have focused on children,[11–18] whereas influenza shedding dynamics in adults remains poorly defined.[19–21] Although adult studies have reported widely varying detection rates of influenza A and B following LAIV, they consistently report lower shedding rates in adults at 4.2%-50.0%,[19–21] compared to the 8.9%-79.0% in pediatric populations.[12–14] Adult studies began sampling later than pediatric studies, suggesting early shedding could be an important part of adult LAIV response that was previously missed.

Vaccine composition may also contribute to the variability in seasonal post-LAIV shedding and vaccine efficacy. LAIV vaccine composition is generally updated annually, possibly resulting in adjustments in strain selection and the number of influenza A and B strains included. Prior studies in adolescent populations suggest vaccine composition can influence shedding patterns;[5,6,16] yet, its impact in adult populations is unknown. Understanding whether and how strain composition

impacts post-vaccination replication is critical for interpreting seasonal variations in LAIV performance and guiding future vaccine design.

To address these gaps, we conducted a study of healthy adults across two influenza seasons, where the 2023-2024 cohort received a quadrivalent LAIV and the 2024-2025 cohort received a trivalent LAIV. We characterized the short-term dynamics of post-vaccination influenza A and B viral RNA shedding starting on the first day post vaccination and assessed concurrent self-reported respiratory symptoms. By linking viral shedding dynamics to vaccine composition and symptom burden, this study provides new insights into LAIV replication in healthy adult populations.

MATERIALS AND METHODS

Study Design and Sample Collection

During the 2023-2024 and 2024-2025 influenza seasons, individuals aged 18–49 years who had elected to receive the live attenuated influenza vaccine (LAIV) and had not yet received an influenza vaccine for the current season were recruited on and around George Washington University's Foggy Bottom campus and screened for eligibility. Exclusion criteria followed CDC LAIV guidelines and included systemic antibiotic or corticosteroid use in the previous two months; immune-related disorders requiring medication; or contraindications to anterior nasal sampling or venous blood draw. Participants that enrolled in the 2023-2024 cohort were not eligible for enrollment in the 2024-2025 cohort. Eligible participants were enrolled in our observational study.

In 2023–2024, participants received the quadrivalent FluMist® vaccine (AstraZeneca) containing approximately $10^{6.5\text{-}7.5}$ fluorescent focus units of each of the four recombinant strains: A/Norway/31694/2022 (H1N1), A/Norway/16606/2021 (H3N2), B/Phuket/3073/2013 (B/Yamagata lineage), and B/Austria/1359417/2021 (B/Victoria lineage).[22] In 2024–2025, participants received the trivalent FluMist® vaccine containing approximately $10^{6.5\text{-}7.5}$ fluorescent focus units of each of the three recombinant strains: A/Norway/31694/2022 (H1N1), A/Perth/722/2024 (H3N2), and B/Austria/1359417/2021 (B/Victoria lineage).[23] Study screening form and participant questionnaire can be found in **Table S1**. A total of 7 participants were excluded due to missing two or more follow-up visits.

Participants self-collected nasal swabs at the baseline visit prior to receiving LAIV and at three additional post-LAIV visits on day 1, day 2-4, and day 5-7. At each study visit, patients completed the Wisconsin Upper Respiratory Symptom Survey (WURSS-11).[24] Swabs were immediately collected into 1 mL of DNA/RNA shield (R1100-250, Zymo), stored at 4°C, and processed within 2 hours. All participants provided written informed consent. The study protocol was approved by the George Washington University Institutional Review Board (NCR234806).

Influenza A and B Detection and Quantification

RNA was extracted from 50 uL of swab eluent using the MagMAX™-96 Viral RNA Isolation Kit (Thermo Fisher) using a final elution volume of 50 uL. Influenza A and B RNA were quantified by RT-qPCR adapted from Boikos et al.,[14] with human ribonuclease P (RP) as an internal extraction control (**Figure S1**). Reactions were performed using the QuantiTect One-Step RT-PCR Kit (Qiagen) on the LightCycler® 480 II (Roche). Each run included plasmid standards, negative template, and negative subject controls. Limits of detection and assay validation procedures are described in the Supplementary Methods.

Data analysis

The primary endpoints were post-LAIV influenza viral detection, duration of viral shedding, and post-vaccination symptoms. Influenza A and B detection and shedding were measured based on RT-qPCR cycle threshold (Ct) values converted to virus copy numbers using in-run standard curves. Continuous variables are presented as medians with interquartile range (IQR), and categorical variables are expressed as frequencies. Proportions and 95% confidence intervals were estimated using a binomial distribution.

Proportions were compared using the two-proportion z-test, Chi-squared test or Fisher exact test, as appropriate, and medians were compared using the Mann-Whitney U test or Kruskal-Wallis test. Predicted probabilities and 95% confidence intervals of influenza detection were estimated using logistic mixed-effects regression models to account for repeated measures. Logistic regression models were used to assess associations between host factors, influenza season, and detection of influenza. Duration of viral shedding was analyzed using Cox proportional hazards models, with results reported as hazard ratios (HRs) and 95% confidence intervals. Linear mixed-effects regression models were used to estimate the viral load decay rate. Interaction terms between the slope (days post-vaccination) and influenza type or codetection status were included to assess whether viral load decay differed by influenza type or codetection status.

Total symptom burden and total viral burden were determined by calculating the area under the curve (AUC) of reported WURSS-11 symptom sum score and viral load over the follow-up period using the trapezoidal rule (**Figure S2**). Linear regression models were used to assess predictors that are associated with total symptom burden. All statistical analyses were conducted in SAS version 9.4, and all figures and graphs were prepared in R version 4.4.2.

RESULTS

Study Population

A total of 283 participants (108 in 2023-2024, 175 in 2024-2025) were included in our analysis. Participants had a median age of 22 years (Interquartile range: 20-26, Range: 18-49) and were

predominantly female (70.7%) and Caucasian (64.1%) (**Table 1**). Most (62.8%) participants had received influenza vaccination within 14 months prior to their enrollment date (**Table 1**). Recent smoking was the only participant characteristic to differ by influenza season (**Table S2**). In the 2023-2024 season, 94.6%, 97.3%, and 92.8% participants completed post-LAIV day 1, day 2-4, and day 5-7 visits, respectively. In the 2024-2025 season, 96.6%, 97.2%, 96.1% participants completed post-LAIV day 1, day 2-4, and day 5-7 visits, respectively. The distribution of RP Ct values did not differ by study visit (**Figure S1**).

Influenza A and B detection following live attenuated influenza vaccination

Within one week of LAIV vaccination, influenza A and B viral RNAs were frequently detected in participants' nasal cavities. Influenza A was detected in 172 (60.8%) participants, and influenza B in 242 (85.6%) participants (**Figure 1A**); overall, 258 (91.2%) participants had either influenza A or B detected within the first week (**Figure 1B**). Co-detection of influenza A and B occurred in 156 (55.1%) participants, whereas 16 (5.7%) had influenza A only and 86 (30.4%) had influenza B only (**Figure 1B**).

Post-LAIV viral detection patterns varied by year of administration. With the quadrivalent vaccine (two influenza A and two influenza B strains) used in the 2023-2024 season, influenza B detection was more common compared to the 2024-2025 season (93.5% vs. 80.6%, $p < 0.01$, **Figure 1A**). In contrast, with the trivalent vaccine (two influenza A strains and one influenza B strain), influenza A detection was more common compared to the 2023-2024 season (66.9% vs. 50.9%, $p = 0.01$, **Figure 1A**). Age**,** sex**,** prior influenza vaccination, and recent smoking status were not significantly associated with post-LAIV influenza detection in either season (**Table 2, Table S3**). These findings suggest that changes in vaccine strain composition across years may impact post-LAIV viral detection.

Duration and burden of Influenza A and B viral shedding following live attenuated influenza vaccination

The duration and burden of viral shedding following LAIV differed significantly between influenza A and B. Shedding duration was longer for influenza B than influenza A (median, 2.0 vs. 1.0 days; $p < 0.001$, **Table S4**). Influenza B also demonstrated a higher overall shedding burden than influenza A throughout the study period ($p < 0.001$), with a median of 4.2 $\log_{10}$ RNA copies per swab on post-LAIV day 1 compared with 4.0 $\log_{10}$ RNA copies for influenza A (**Table S4**).

Peak viral shedding occurred on post-LAIV day 1 for both virus types, with 82.0% and 72.6% of participants exhibiting peak influenza A and B shedding, respectively. By the post-LAIV day 2-4 visit, influenza A was detected in only 16.8% of participants, whereas influenza B remained detectable in 53.2% of participants. By the post-LAIV day 5-7 visit, influenza A detection decreased to 4.0%, while influenza B remained detectable in 15.7% of participants (**Figure 1C**). These findings demonstrate greater replication and persistence of Influenza B in the upper respiratory tract than influenza A following LAIV.

Post-LAIV viral shedding patterns varied by influenza season. During the 2023-2024 season (quadrivalent vaccine), influenza A and influenza B exhibited a higher peak viral shedding burden than the 2024-2025 season ($p < 0.001$, **Table S4**). Viral shedding was higher with the quadrivalent vaccine for both influenza A and B, but this difference was only observed on post-LAIV day 1. The total viral shedding burden was also higher for influenza B when a quadrivalent vaccine was used ($p < 0.001$, **Table S4**), but did not differ for influenza A. These findings suggest that changes in vaccine strain composition across years may impact other viral shedding outcomes such as peak and total viral shedding burden post-LAIV.

Influenza A and B viral clearance following live attenuated influenza vaccination

Viral clearance rate differed significantly between influenza A and B, with a 62% faster clearance rate for influenza A compared with influenza B (adjusted hazard ratio 1.62, 95% CI 1.29-2.03, **Table S5**). This difference was reflected in the distribution of three distinct viral clearance phenotypes: slow (viral shedding for >= 7 days post-LAIV), moderate (viral shedding through 2-4 days post-LAIV, clearance by day 5-7), and rapid (clearance within 2-4 days post-LAIV). Total viral burden varied significantly by viral clearance profile (median = 3.74 for rapid; 14.5 for moderate; 22.6 for low). Age, sex, prior influenza vaccination, and recent smoking status were not associated with viral clearance profiles (**Table S6**).

The distribution of viral clearance profiles differed by influenza type (χ^2 $p < 0.001$, **Table S4**). Influenza B clearance profiles were more likely to be slow (15.7% vs. 5.0%, p=0.001) and moderate (47.5% vs. 20.6%, p<0.001) compared with influenza A and less likely to be rapid (36.8% vs. 74.4%, p<0.001). Viral decay rates differed across the three viral clearance profiles (slow vs. moderate vs. fast) but did not differ by influenza type or codetection status, suggesting that decay patterns align closely with clearance phenotype classification (**Figure 2, Table S7**). In contrast to the significant variations in detection rates and peak viral shedding burden between 2023-2024 and 2024-2025 seasons, viral shedding duration and the distribution of viral clearance profiles for influenza A and influenza B were consistent across years (**Table S4**).

Probability of viral detection by day following live attenuated influenza vaccination

To characterize the temporal dynamics of post-LAIV viral replication, we modeled the probability of influenza RNA detection by day following vaccination. Model-based estimates showed a rapid decline in detection probability after vaccination. The predicted probability of detecting influenza A or B RNA was 92% (95% CI, 88%–94%) on day 1, 23% (17%-30%) on day 5, and 9% (5%–13%) by day 7 (**Table 3**). Influenza B had higher day-1 predicted detection (89% vs. 59% for influenza A) and a slower decline across timepoints (**Figure 1D**). These findings indicate that viral replication begins promptly after vaccination but decreases sharply thereafter, with most adults clearing detectable RNA within the first week.

Association between viral shedding and respiratory symptoms following live attenuated influenza vaccination.

Mild respiratory symptoms were common during post-LAIV follow-up visits. The most common symptoms were runny nose (58.3%), feeling tired (58.0%), and nasal congestion (50.2%), while sore throat (27.9%), scratchy throat (24.0%) and itchy nose (20.8%) were the least common (**Table S8**). The median total symptom burden at the baseline vaccination visit was 1.0 (Q1: 0.0, Q3: 3.0). Symptom patterns varied across visits, with feeling tired (40.7%) being the most common on post-LAIV day 1, runny nose (41.8%) being the most common during days 2-4 and feeling tired (33.5%) again the most common during days 5-7 (**Table S8**). The median total symptom burden was 12.8 (Q1: 5.0, Q3: 30.8), with median scores of 17.2 and 15.0 for those with influenza A, and influenza B detected, respectively. Symptom burden differed for those with and without virus detected ($p<0.01$, **Figure S3**). Symptom burden was higher among those with influenza A detected compared to influenza B (**Figure S4**). Total symptom burden did not differ between participants with detectable influenza A or B across influenza seasons ($p = 0.42$, **Table S9**).

Among the viral shedding parameters, total viral shedding burden and total duration of viral shedding were the strongest predictors of total symptom burden (**Table 4**). Although influenza A exhibited a shorter duration of viral shedding and lower total viral burden than influenza B (**Table S4, Figure S5**), its shedding duration was more strongly associated with symptom burden (**Table S10**). These findings indicate that greater viral replication and prolonged shedding contribute to increased respiratory symptom burden following LAIV administration, and that influenza A strains may have a greater impact on symptom burden.

DISCUSSION

To our knowledge, this is the first study to characterize early post-LAIV shedding in an adult population. We found that post-LAIV viral shedding in adults may be comparable to those observed in pediatric populations. Specifically, 91% of adults in our study had detectable influenza A or B RNA after LAIV, in contrast to prior reports showing 4.2%-50.0% detection of influenza viral RNA in adults[19–21]. This likely reflects earlier sampling, short shedding duration, differences in vaccine strain composition,[5,6,16] and more sensitive RT-qPCR detection[25] compared to immunofluorescence or tissue culture used in earlier studies.[19–21] Given that post-LAIV shedding has been linked to the magnitude of immune responses,[9,10] the higher shedding rate we observed in adults may provide a biological explanation for why LAIV efficacy in adults can be similar to that observed in children.

Vaccine composition may impact post-vaccination viral RNA detection and shedding patterns.

Reducing influenza B strains from two (2023-2024) to one (2024-2025) coincided with higher rates of influenza A shedding and lower rates of influenza B shedding. Because early mucosal viral

dynamics have been shown to influence downstream systemic antibody responses,[10] differences in replication driven by strain composition may have immunologic relevance. Further studies are needed to determine how vaccine composition impacts post-LAIV shedding and immunity, and how these outcomes interact with baseline pre-existing immunity.

We also observed that post-LAIV viral shedding was associated with symptom burden in adults, similar to what has been reported in children and adults with RT-qPCR-confirmed influenza infections.[26,27] Influenza A typically causes more severe illness than influenza B among children and adolescents,[28] which may also explain why we observed a significant association between symptom burden and influenza A shedding patterns, but not influenza B.

Consistent with earlier studies,[15,16,19,21] influenza B was detected more frequently than influenza A after LAIV and was associated with more persistent and higher levels of viral shedding. By combining frequency, duration, and shedding levels, we observed three distinct viral patterns - slow, moderate and rapid - that were applicable to both influenza types. Influenza A was predominantly associated with the rapid clearance profile, while influenza B was more frequently associated with moderate and slow clearance profiles. These patterns likely reflect the presence of coordinated nasal immune programs that include contributions from local and systemic pre-existing and innate immunity. Community influenza circulation in preceding years may further modulate these responses; for example, the overwhelming predominance of influenza A in the 2021–2022 and 2022–2023 U.S. seasons could have increased baseline anti-A immunity and suppressed LAIV-A replication.

Our model-based estimates of the probability of viral RNA detection by day post-LAIV support that detection was highly likely at day 1 (92%) but declined steeply over time, reaching 23% by day 5 and 9% by day 7. These dynamics reinforce that LAIV replication begins rapidly after vaccination but is short-lived in most adults. Although clinical transmission from vaccine recipients has not been demonstrated in real-world settings and ACIP considers the risk of onward transmission to be theoretical and relevant primarily to close contacts of severely immunocompromised individuals, the rapid decrease in detection probability we observed suggests that, even if secondary transmission were possible, opportunities would be largely restricted to the earliest days following vaccination.[29]

Our study had several limitations. Our study population was composed primarily of younger, white, and female adults which could limit the generalizability of our study. We also have not characterized baseline immune status of our study population yet, which has been shown to influence post-LAIV shedding.[20,30,31] This limitation is to be addressed in further studies, as characterization of baseline immune status is underway. Additionally, while RT-qPCR is routinely used to as a clinical diagnostic for influenza and to assess LAIV nasal shedding post-vaccination in research studies,[14–17,26] PCR-based methods cannot specifically discriminate between infectious virions and non-infectious viral nucleic acids. This ultimately limits our ability to draw specific conclusions related to contagiousness of LAIV vaccines. Further, early detection may partially

reflect residual vaccine virus; nonetheless, the subsequent shedding kinetics and subtype-specific patterns indicate active replication rather than passive persistence alone. Despite these limitations, our findings provide the most detailed characterization to date of early post-LAIV shedding in adults, which can have important implications for our understanding of the mucosal vaccine efficacy in the adult population.

In summary, we found that post-LAIV viral shedding in a healthy adult population is substantially more common than previously reported and is similar to rates in pediatric populations. In particular, influenza A shedding was a significant determinant of post-LAIV respiratory symptoms. Further studies are needed to understand how post-LAIV viral shedding dynamics, including the three distinct viral shedding profiles observed in this study, impact LAIV efficacy, and to determine vaccine compositions that best balance viral shedding, symptom burden, transmission risk, and protective immunogenicity.

Data Availability: The data underlying this article will be shared on reasonable request to the corresponding author.

Acknowledgments: We would like to thank all the participants of our study that generously volunteered their time and effort. We would also like to thank Megan Spyers-Duran, Jade Ditta, Skylar Adderley, Lauren Knight, Marwa Hameed, Isabela Burton, and Stephanie Metis for their time, effort, and support with sample processing. The successful completion of this publication would not have been possible without the contributions of all participants and lab staff.

Funding: This work was supported by the National Institutes of Health [grant number 5R01AI168182-03] and a Research Enhancement Award from the Office of Research Excellence, Milken Institute School of Public Health, George Washington University, USA.

Potential conflicts of interest: The authors of this manuscript do not have a commercial or other association that might pose a conflict of interest.

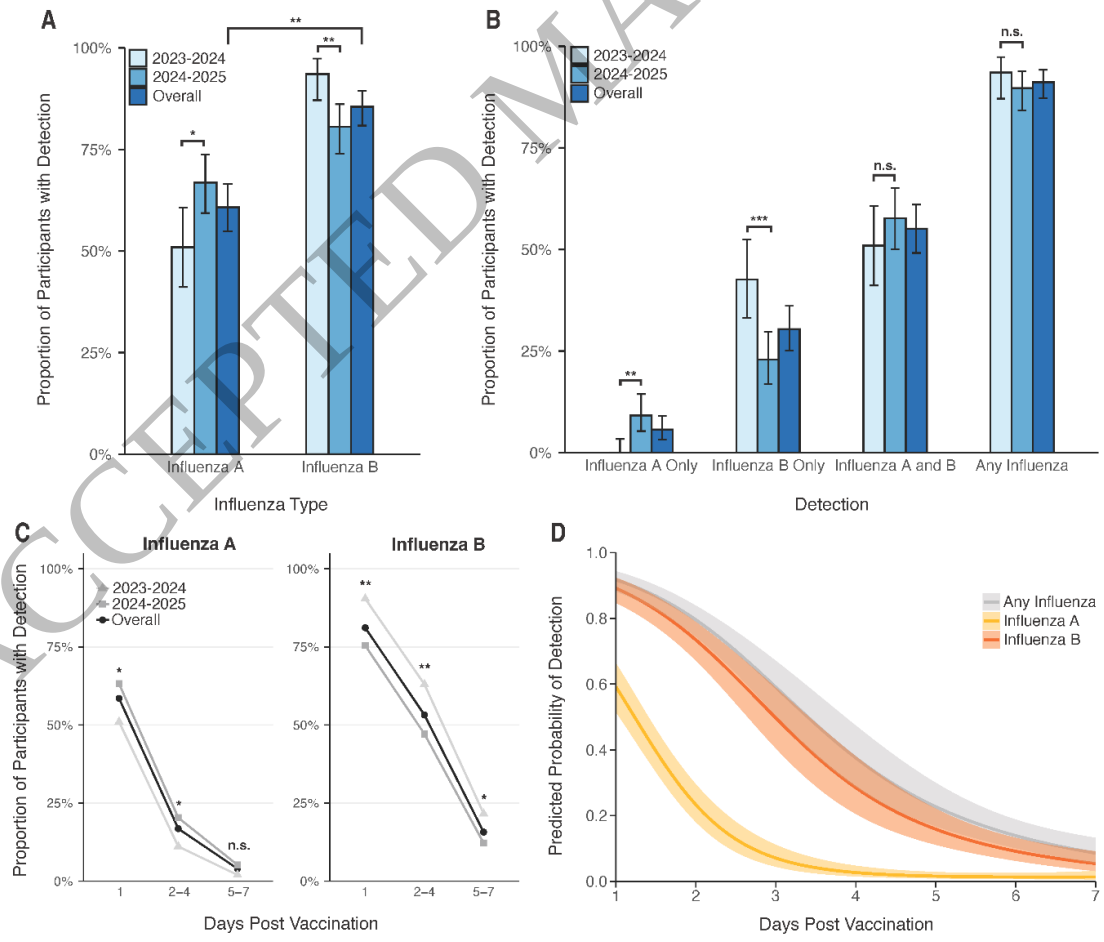
References

1. Kalarikkal SM, Jaishankar GB. Influenza Vaccine. In: *StatPearls*. StatPearls Publishing; 2025. Accessed August 19, 2025. http://www.ncbi.nlm.nih.gov/books/NBK537197/
2. Using Live, Attenuated Influenza Vaccine for Prevention and Control of Influenza. Accessed October 18, 2025. https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5213a1.htm
3. Hoft DF, Babusis E, Worku S, et al. Live and Inactivated Influenza Vaccines Induce Similar Humoral Responses, but Only Live Vaccines Induce Diverse T-Cell Responses in Young Children. *J Infect Dis*. 2011;204(6):845-853. doi:10.1093/infdis/jir436
4. Mohn KGI, Smith I, Sjursen H, Cox RJ. Immune responses after live attenuated influenza vaccination. *Hum Vaccin Immunother*. 2018;14(3):571-578. doi:10.1080/21645515.2017.1377376
5. Bandell A, Kassianos G, Dibben O, El Azzi G. Comparative effectiveness of live attenuated influenza vaccine (LAIV) and inactivated influenza vaccine (IIV) in children over multiple influenza seasons (2019–2023). *Vaccine: X*. 2025;25:100666. doi:10.1016/j.jvacx.2025.100666

6. Chung JR, Flannery B, Thompson MG, et al. Seasonal Effectiveness of Live Attenuated and Inactivated Influenza Vaccine. *Pediatrics*. 2016;137(2):e20153279. doi:10.1542/peds.2015-3279
7. Ambrose CS, Levin MJ, Belshe RB. The relative efficacy of trivalent live attenuated and inactivated influenza vaccines in children and adults. *Influenza Other Respir Viruses*. 2011;5(2):67-75. doi:10.1111/j.1750-2659.2010.00183.x
8. Hoft DF, Lottenbach KR, Blazevic A, et al. Comparisons of the Humoral and Cellular Immune Responses Induced by Live Attenuated Influenza Vaccine and Inactivated Influenza Vaccine in Adults. *Clinical and Vaccine Immunology*. 2017;24(1):e00414-16. doi:10.1128/CVI.00414-16
9. Matrajt L, Halloran ME, Antia R. Successes and Failures of the Live-attenuated Influenza Vaccine: Can We Do Better? *Clinical Infectious Diseases*. 2020;70(6):1029-1037. doi:10.1093/cid/ciz358
10. Thwaites RS, Uruchurtu ASS, Negri VA, et al. Early mucosal events promote distinct mucosal and systemic antibody responses to live attenuated influenza vaccine. *Nat Commun*. 2023;14(1):8053. doi:10.1038/s41467-023-43842-7
11. King JCJ, Fast PE, Zangwill KM, et al. Safety, vaccine virus shedding and immunogenicity of trivalent, cold-adapted, live attenuated influenza vaccine administered to human immunodeficiency virus-infected and noninfected children. *The Pediatric Infectious Disease Journal*. 2001;20(12):1124.
12. Mallory RM, Yi T, Ambrose CS. Shedding of Ann Arbor strain live attenuated influenza vaccine virus in children 6–59 months of age. *Vaccine*. 2011;29(26):4322-4327. doi:10.1016/j.vaccine.2011.04.022
13. Levin MJ, Song LY, Fenton T, et al. Shedding of Live Vaccine Virus, Comparative Safety, and Influenza-Specific Antibody Responses after Administration of Live Attenuated and Inactivated Trivalent Influenza Vaccines to HIV-Infected Children. *Vaccine*. 2008;26(33):4210-4217. doi:10.1016/j.vaccine.2008.05.054
14. Boikos C, Joseph L, Martineau C, et al. Influenza Virus Detection Following Administration of Live-Attenuated Intranasal Influenza Vaccine in Children With Cystic Fibrosis and Their Healthy Siblings. *Open Forum Infectious Diseases*. 2016;3(4):ofw187. doi:10.1093/ofid/ofw187
15. Dar L, Krishnan A, Kumar R, et al. Nasal shedding of vaccine viruses after immunization with a Russian-backbone live attenuated influenza vaccine in India. *Influenza and Other Respiratory Viruses*. 2023;17(6):e13149. doi:10.1111/irv.13149
16. Jackson D, Pitcher M, Hudson C, et al. Viral Shedding in Recipients of Live Attenuated Influenza Vaccine in the 2016–2017 and 2017–2018 Influenza Seasons in the United Kingdom. *Clinical Infectious Diseases*. 2020;70(12):2505-2513. doi:10.1093/cid/ciz719
17. Lewis KDC, Ortiz JR, Rahman MZ, et al. Immunogenicity and Viral Shedding of Russian-Backbone, Seasonal, Trivalent, Live, Attenuated Influenza Vaccine in a Phase II, Randomized, Placebo-Controlled Trial Among Preschool-Aged Children in Urban Bangladesh. *Clinical Infectious Diseases*. 2019;69(5):777-785. doi:10.1093/cid/ciy1003
18. Brickley EB, Wright PF, Khalenkov A, et al. The Effect of Preexisting Immunity on Virus Detection and Immune Responses in a Phase II, Randomized Trial of a Russian-Backbone, Live, Attenuated Influenza Vaccine in Bangladeshi Children. *Clinical Infectious Diseases*. 2019;69(5):786-794. doi:10.1093/cid/ciy1004
19. King JC Jr, Treanor J, Fast PE, et al. Comparison of the Safety, Vaccine Virus Shedding, and Immunogenicity of Influenza Virus Vaccine, Trivalent, Types A and B, Live Cold-Adapted,

Administered to Human Immunodeficiency Virus (HIV)-Infected and Non-HIV-Infected Adults. *The Journal of Infectious Diseases*. 2000;181(2):725-728. doi:10.1086/315246
20. Block SL, Yogev R, Hayden FG, Ambrose CS, Zeng W, Walker RE. Shedding and immunogenicity of live attenuated influenza vaccine virus in subjects 5–49 years of age. *Vaccine*. 2008;26(38):4940-4946. doi:10.1016/j.vaccine.2008.07.013
21. Talbot TR, Crocker DD, Peters J, et al. Duration of virus shedding after trivalent intranasal live attenuated influenza vaccination in adults. *Infect Control Hosp Epidemiol*. 2005;26(5):494-500. doi:10.1086/502574
22. MedImmune, LLC. FluMist® Quadrivalent [package insert]. U.S. Food and Drug Administration; August 2023. Available at: https://www.fda.gov/media/160349/download.
23. MedImmune, LLC. FluMist® Trivalent [package insert]. U.S. Food and Drug Administration; August 2025. Available at: https://www.fda.gov/media/180697/download.
24. Obasi CN, Brown RL, Barrett BP. Item Reduction of the Wisconsin Upper Respiratory Symptom Survey (WURSS-21) Leads to the WURSS-11. *Qual Life Res*. 2014;23(4):1293-1298. doi:10.1007/s11136-013-0561-z
25. Sung RYT, Chan PKS, Choi KC, et al. Comparative Study of Nasopharyngeal Aspirate and Nasal Swab Specimens for Diagnosis of Acute Viral Respiratory Infection. *J Clin Microbiol*. 2008;46(9):3073-3076. doi:10.1128/JCM.01209-08
26. Wang B, Russell ML, Fonseca K, et al. Predictors of influenza a molecular viral shedding in Hutterite communities. *Influenza and Other Respiratory Viruses*. 2017;11(3):254-262. doi:10.1111/irv.12448
27. McKay B, Ebell M, Billings WZ, Dale AP, Shen Y, Handel A. Associations Between Relative Viral Load at Diagnosis and Influenza A Symptoms and Recovery. *Open Forum Infect Dis*. 2020;7(11):ofaa494. doi:10.1093/ofid/ofaa494
28. Jané M, Vidal MJ, Soldevila N, et al. Epidemiological and clinical characteristics of children hospitalized due to influenza A and B in the south of Europe, 2010–2016. *Sci Rep*. 2019;9:12853. doi:10.1038/s41598-019-49273-z
29. Grohskopf LA. Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices — United States, 2025–26 Influenza Season. *MMWR Morb Mortal Wkly Rep*. 2025;74. doi:10.15585/mmwr.mm7432a2
30. Bean R, Giurgea LT, Han A, et al. Mucosal correlates of protection after influenza viral challenge of vaccinated and unvaccinated healthy volunteers. *mBio*. 15(2):e02372-23. doi:10.1128/mbio.02372-23
31. Gould VMW, Francis JN, Anderson KJ, Georges B, Cope AV, Tregoning JS. Nasal IgA Provides Protection against Human Influenza Challenge in Volunteers with Low Serum Influenza Antibody Titre. *Front Microbiol*. 2017;8:900. doi:10.3389/fmicb.2017.00900

Figure 1. Detection rates and probability of detection of influenza A and B following live attenuated influenza vaccination. (A) Overall rate of RT-qPCR–confirmed influenza B detection (86.6%) was significantly higher than influenza A detection (61.5%) ($p < 0.001$). Influenza A detection was significantly higher in the 2024-2025 season compared to the 2023-2024 season (66.9% vs. 50.9%, $p < 0.05$). Influenza B detection was significantly higher in the 2023-2024 season compared to the 2024-2025 season (93.5% vs. 80.6%, $p < 0.01$) (B) Seasonal differences were observed for influenza A only and influenza B only, while co-detection (influenza A and B) and overall influenza detection did not differ significantly by season. (C) Influenza B was detected at higher rates than influenza A at all post-vaccination visits. Influenza A was detected at higher rates at post-LAIV day 1 and day 2-4 in the 2024-2025 season. Influenza B was detected at higher rates at all study timepoints in the 2023-2024 season. (D) Predicted probabilities from logistic mixed-effects regression models, estimated among all participants, show distinct detection probabilities for influenza A and B. The probability of influenza A detection declined more rapidly, whereas the probability of influenza B detection remained higher than influenza A and very similar to the probability of any influenza detection. Shaded regions represent 95% confidence intervals. All statistical tests comparing proportions used the two-proportion z-test. Asterisks represent significant p-values (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).



DOI: 10.1093/cid/ciag294

Figure 2. Viral clearance profiles of influenza A and B following live attenuated influenza vaccination. Spaghetti plots show viral shedding over three post-vaccination intervals (post-LAIV day 1, day 2–4, and day 5–7) in participants with RT-qPCR–confirmed influenza A or B, stratified by influenza type and viral clearance profile. Gray lines represent individual participants, and blue lines indicate group-level trends. Slope and conditional R^2 for each linear mixed-effect model are reported in black text. Sample size of each viral clearance profile is reported in black text.

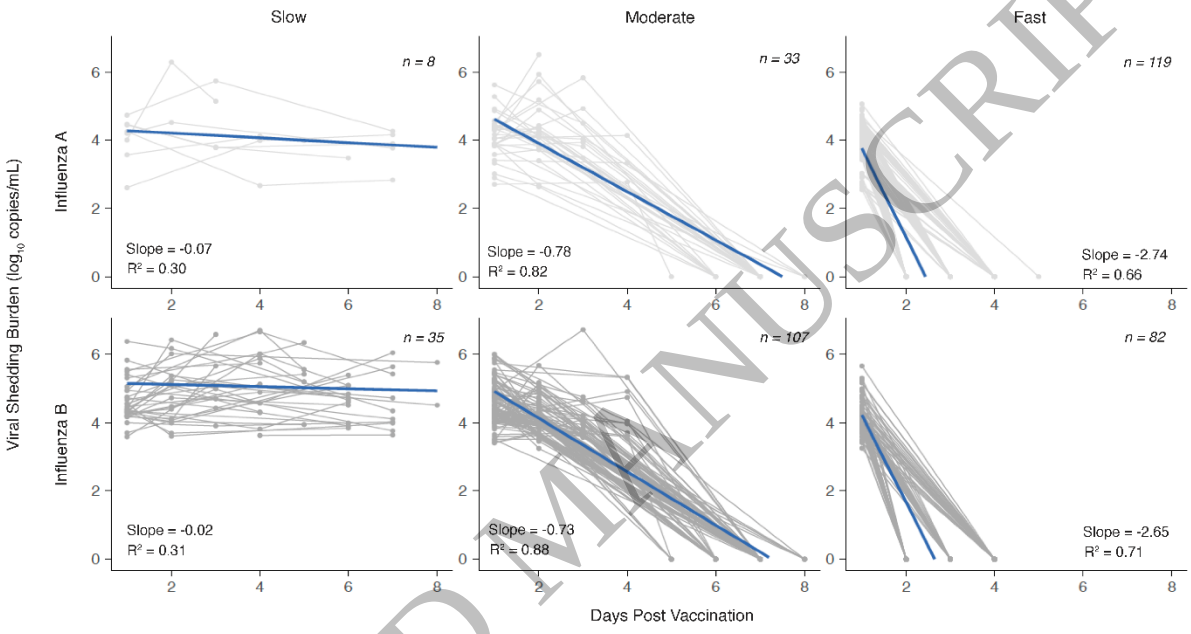


Table 1. Baseline characteristics of study participants

Characteristic	No. (%)
Sex	
Female	202 (71.4%)
Male	81 (28.6%)
Age, years	
Age, median (Q1-Q3)	23 (20-26)
Range	18 - 49
Race	
Caucasian	183 (64.7%)
Black or African American	31 (11.0%)
Asian	47 (16.6%)
Multiracial	13 (4.6%)
Missing	9 (3.2%)
Ethnicity	
Hispanic or Latino	39 (13.8%)
Not Hispanic or Latino	244 (86.2%)

Influenza Vaccination[a]	
Yes	177 (62.5%)
No	105 (37.1%)
Missing	1 (0.4%)
Recent Smoking[b]	
Yes	51 (18.0%)
No	232 (82.0%)
Environmental Allergies	
Yes	141 (49.8%)
No	141 (49.8%)
Missing	1 (0.4%)

[a] Defined as receipt of an influenza vaccine within 14 months prior to their enrollment

[b] Defined as use of a cigarette, e-cigarette, cigar, or hookah within 30 days of enrollment

Table 2. Association of influenza A and B detection with influenza season and host factors

	Influenza A			Influenza B		
Variable	n/N (%)	aOR[a] (95% CI)	p-value[b]	n/N (%)	aOR[a] (95% CI)	p-value[b]
Influenza Season						
2023-2024	55/108 (50.9%)	0.51 (0.31, 0.84)	**0.01**	101/108 (93.5%)	3.38 (1.43, 8.00)	**0.01**
2024-2025	117/175 (66.9%)	Ref	-	141/175 (80.6%)	Ref	-
Influenza Vaccination						
Yes	106/177 (59.9%)	0.90 (0.54, 1.50)	0.68	152/177 (85.9%)	0.99 (0.49, 2.02)	0.98
No	66/105 (62.9%)	Ref	-	89/105 (84.8%)	Ref	-
Recent Smoking						
Yes	33/51(64.7%)	1.09 (0.56, 2.10)	0.81	40/51(78.4%)	0.67 (0.30, 1.51)	0.34
No	139/232 (59.9%)	Ref	-	202/232 (87.1%)	Ref	-
Sex						

Female	126/202 (62.4%)	1.27 (0.74, 2.19)	0.39	174/202 (86.1%)	1.22 (0.58, 2.57)	0.60
Male	46/81 (56.8%)	Ref	-	68/81(84.0%)	Ref	-
Age	-	1.00 (0.96, 1.05)	0.83	-	1.02 (0.96, 1.08)	0.58

Abbreviations: aOR, adjusted odds ratio, CI, confidence interval
Bold p-values represent statistical significance at the $p < 0.05$ level
[a] Multivariate logistic regression adjusted for all variables in table

Table 3. Predicted probability of influenza detection by day post vaccination

	Influenza A	Influenza B	Any Influenza
Day Post Vaccination	Mean Predicted Probability (95% CI)[a]	Mean Predicted Probability (95% CI)[a]	Mean Predicted Probability (95% CI)[a]
1	0.59 (0.52, 0.66)	0.89 (0.85, 0.92)	0.92 (0.88, 0.94)
2	0.23 (0.18, 0.30)	0.73 (0.67, 0.79)	0.80 (0.74, 0.84)
3	0.07 (0.04, 0.11)	0.50 (0.40, 0.59)	0.59 (0.51, 0.67)
4	0.03 (0.01, 0.05)	0.28 (0.21, 0.38)	0.38 (0.29, 0.47)
5	0.02 (0.01, 0.03)	0.16 (0.11, 0.22)	0.23 (0.17, 0.30)
6	0.01 (0.01, 0.03)	0.09 (0.06, 0.13)	0.14 (0.10, 0.19)
7	0.01 (0.01, 0.03)	0.05 (0.03, 0.09)	0.09 (0.05, 0.13)

Abbreviations: CI, confidence interval
[a]Predicted probabilities are model-based estimates from a logistic mixed-effects regression model, and represent predicted probabilities for all study participants

Table 4. Viral determinants of respiratory symptoms

Variable	Estimate[a] (95% CI)	p-value
Total Viral Burden[b]	0.42 (0.15, 0.70)	**0.003**
Total Duration of Viral Shedding[b]	1.65 (0.39, 2.91)	**0.01**
Total Peak Viral Load[b]	0.41 (-0.56, 1.38)	0.41
Influenza A Clearance Profile		
Fast	-8.19 (-18.11, 1.72)	0.10
Slow	1.97 (-17.88, 21.82)	0.84
Moderate	Ref	-
Influenza B Clearance Profile		
Fast	-4.10 (-11.45, 3.25)	0.27
Slow	-1.10 (-10.83, 8.64)	0.82
Moderate	Ref	-

Abbreviations: CI, confidence interval

Bold p-values represent statistical significance at the $p < 0.05$ level.

[a] Multivariate linear regression adjusting for age, sex, influenza vaccination, and environmental allergies

[b] Total viral determinants were calculated by summing total viral burden (AUC) for influenza A and B for each participant