DOI: https://doi.org/10.70749/ijbr.v3i8.2648

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://ijbr.com.pk
ISSN: 2960-2793/ 2960-2807

Viral Shedding Dynamics in Vaccinated Vs Unvaccinated Populations with New Influenza Variants

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ARTICLE INFO

Keywords: Influenza vaccine, Viral shedding, Viral dynamics, Cycle threshold (Ct) value, Vaccine effectiveness, Infectious duration, Influenza variants, Clinical severity

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Declaration
Authors' Contribution: All authors equally contributed to the study and approved the final manuscript.

Conflict of Interest: No conflict of interest.

Funding: No funding received by the authors.

Article History
Received: 12-06-2025 Revised: 14-08-2025
Accepted: 18-08-2025 Published: 30-08-2025

ABSTRACT

Background: Effectiveness of the annual influenza vaccine is mostly quantified by severe disease prevention but its role in influenza shedding dynamics and the period of infectivity, in particular against circulating variants, is a concern of critical importance in understanding the public health. The purpose of the present study was to compare the viral load, shedding period, and the severity of the clinical condition of vaccinated and unvaccinated adults with confirmed influenza infection. **Methods:** The participants in this observational cohort study (n=72) were evenly divided into two groups based on whether they were vaccinated or not with the influenza virus through laboratory tests (n=36 vaccinated and n=36 unvaccinated). There was close matching of participants in terms of baseline. Nares pharyngeal swabs were taken on a daily basis up to 10 days or until two consecutive negative RT-PCR results were achieved. The results were viral load (Ct values), viral decline rate, total shedding time, culture positive and measures of clinical severity (duration of symptoms and incidence of moderate-severe illness). **Results:** There was a similarity between groups in terms of baseline characteristics (p > 0.42 all). Viral control was better in the vaccinated group where both the baseline and peak viral load was significantly lower (p=0.002 and p=0.004) and the rate of viral decline was also much faster (2.8 +- 0.6 Ct/day vs 1.9 +- 0.5 Ct/day, p<0.001). Most importantly, there was an association between vaccination and decreased Total Shedding Duration (4.8 ± 1.3 vs 7.1 ± 1.8 days, p<0.001), and there was less culture positive on Day 5 (19.4% vs 61.1%, p<0.001). There was a significantly long period of symptoms and lower occurrence of moderate-to-severe illness amongst the vaccinated participants (16.7% vs 38.9%, p=0.03). The shielding property on shedding time was strongly seen in all three variants experimented. **Conclusion:** Vaccination against influenza has a great influence on reducing the depth and durability of the illness resulting in a reduced viral load and a considerably reduced period of contagion. These results support the importance of the vaccine to minimize the personal disease burden, as well as the possibility of infection of the community, even with the presence of new influenza strains.

INTRODUCTION

Influenza is one of the significant global health issues that lead to seasonal outbreaks, which cause millions of people to be hospitalized every year and hundreds of thousands die every year across the world. Annual vaccination is the major intervention that has been found to reduce this disease burden, the main objective of which is to prevent severe illness, hospitalization, and death. Nevertheless, the immunity that is produced in response to vaccination, especially in relation to new and drifted influenza strains like H3N2 and Reassortant strains, is not necessarily able to protect against infection in all cases. In the case of breakthrough infections, it is important to know the level of vaccination in impacting on the viral dynamics in the host [1-4].

The severity of illness and the potential for transmission are directly linked to the kinetics of viral replication and the subsequent duration of viral shedding. Viral load, commonly quantified by the Reverse Transcription-Polymerase Chain Reaction (RT-PCR) cycle threshold (Ct) value, provides an indirect measure of the amount of virus present, with lower Ct values indicating higher viral burden. The duration for which an individual sheds viable (culture-positive) virus is a strong proxy for their infectivity window. Understanding how vaccination impacts these specific metricsviral load and the duration of shedding is essential for developing precise isolation guidelines and evaluating the vaccine's benefit in controlling community spread [5-7]

Previous studies have indicated that prior immunity, whether from vaccination or natural infection, can modify the course of subsequent influenza episodes. However, comprehensive data directly comparing the full viral shedding kinetics including baseline load, peak load, time to peak, and rate of decline alongside variant-specific outcomes in a carefully matched cohort are less common. This research addresses this gap by meticulously tracking the full course of infection in vaccinated and unvaccinated individuals exposed to contemporary influenza variants [8-10].

Thus, the aim of the proposed study consisted in a head-to-head comparison of the dynamics of viral shedding (viral load and shedding duration) and clinical severity in the group of adults with laboratory-confirmed influenza who received and did not receive annual influenza vaccinations to measure the biological and clinical advantages of vaccination.

METHODOLOGY

This was an observational cohort study conducted over a one-year period, spanning from March 2024 to February 2025, at Jinnah Hospital, Lahore. The primary objective was to compare viral dynamics, shedding duration, and clinical severity between vaccinated and unvaccinated individuals with confirmed influenza infection.

The total number of individuals that formed the study population was 72 adults with laboratory-confirmed influenza infection who were evenly stratified into two groups:

- Vaccinated Group (n=36): Individuals who had received at least one dose of the current seasonal influenza vaccine.
- Unvaccinated Group (n=36): Individuals who had not received the current seasonal influenza vaccine.

Inclusion and Exclusion Criteria

Inclusion Criteria: The participants had to be 18 years old and older, and the participants had to be presented within 48 hours of the appearance of influenza symptoms. There were also the asymptomatic contacts identified through routine screening.

Exclusion Criteria: The persons with chronic immunodeficiency disorders, people who have started antiviral treatment before the baseline sample were collected, and those with missing follow up data were excluded to maintain consistency of estimates of viral shedding.

Nares and pharyngeal swabs were picked at the baseline (first visit) and after that with a certain interval of 24 hours. This process was repeated up to a maximum of ten days or until the participant gave two consecutive negative Reverse Transcription-Polymerase Chain Reverse Transcription-Polymerase Chain Reaction results.

- Viral Load Dynamics: Viral load was quantified using RT-PCR cycle threshold Ct values. A lower Ct value indicated a higher viral load.
- Viruses in shedding: The viable presence of the virus was evaluated by the presence of viral culture in the swab channel selection days. Duration of Viral Shedding was determined as the interval between the first positive RT-PCR and the first two negative RT-PCRs.

The baseline samples were also typed in terms of influenza variant (H3N2, H1N1, and reassortant strains) by using the validated real-time genomic surveillance panels.

All participants were carefully followed regarding the important clinical outcomes, such as the duration of the symptoms, fever course, cough, and general clinical severity (according to mild, moderate, or severe). Vaccination information, such as the quantity of the doses, the duration since the last dose, and the type of the vaccine were collected systematically through interviewing the patients and comparing them with the official immunization records.

All the primary data were independently extracted by two independent investigators to reduce the possibility of extraction bias. Any difference between the extracted datasets was sorted out by the requirement to discuss and then verify it against the original primary clinical records.

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RESULTS

This table validates that the vaccinated and non-vaccinated groups were not dissimilar in all the baseline demographics, such as age, gender, and the acquisition of major comorbidities (diabetes, hypertension and Asthma/COPD). The values of all p-values were 0.42 which showed that there were no statistically significant differences between the groups in any of the assessed variables. This important observation indicates that differences in the results as noticed can probably be explained by the vaccination as opposed to underlying differences in the characteristics of the patients.

Table 1

Baseline Demographic Characteristics (n = 72)

Variable	Vaccinated (n=36)	Unvaccinated (n=36)	p-value
Age (years), Mean ± SD	38.4 ± 12.1	40.7 ± 11.6	0.42
Gender (Male), n (%)	18 (50.0%)	17 (47.2%)	0.81
Diabetes, n (%)	6 (16.7%)	8 (22.2%)	0.54
Hypertension, n (%)	5 (13.9%)	7 (19.4%)	0.52
Asthma/COPD, n (%)	4 (11.1%)	6 (16.7%)	0.50

The analysis of viral load dynamics shows that the vaccinated group had significantly higher Ct values (indicating lower viral load) both at baseline and at peak infection compared to the unvaccinated group ($p=0.002$ and $p=0.004$, respectively). Furthermore, the vaccinated group exhibited a significantly faster rate of viral decline (2.8 ± 0.6 Ct/day vs 1.9 ± 0.5 Ct/day $p<0.001$) and a slightly longer, yet statistically significant, time to reach peak viral load (2.1 ±0.9 days vs 1.7 ± 0.8/days $p=0.03$). These differences strongly suggest that vaccination helps the immune system to control viral replication more effectively and clear the virus more quickly.

Table 2

Viral Load Dynamics (Ct Values) Between Groups

Variable	Vaccinated (n=36)	Unvaccinated (n=36)	p-value
Baseline Viral Load (Ct), Mean ± SD	27.8 ± 5.1	23.9 ± 4.7	0.002
Peak Viral Load (Ct), Mean ± SD	25.2 ± 4.9	21.7 ± 4.4	0.004
Time to Peak Viral Load (days)	2.1 ± 0.9	1.7 ± 0.8	0.03
Rate of Viral Decline (Ct/day)	2.8 ± 0.6	1.9 ± 0.5	<0.001

Vaccination also showed a very shortened span of total viral shedding and the vaccinated group shed the virus in an average of 4. days as opposed to 7.1 days in the unvaccinated group (p<0.001). This clinical point is made by the fact that there was considerably less shedding (>7 days, 13.9% vs 50.0%, p=0.001) or even culture positive and potentially infectious on Day 5 (19.4 % vs 61.1 %, p<0.001) in vaccinated persons. This table shows a strong preventive action of the vaccine to curb the infectious period and the general duration of time an individual carries the viable virus.

Table 3

Duration of Viral Shedding (Key Outcome)

Variable	Vaccinated (n=36)	Unvaccinated (n=36)	p-value
Total Shedding Duration (days), Mean ± SD	4.8 ± 1.3	7.1 ± 1.8	<0.001
Prolonged Shedding (>7 days), n (%)	5 (13.9%)	18 (50.0%)	0.001
Culture Positive on Day 5, n (%)	7 (19.4%)	22 (61.1%)	<0.001

According to the findings, the vaccination has a much milder effect on the clinical course of the infection, in particular, the number of days of the symptom. In particular, the vaccinated patients experienced significantly reduced fever and cough periods with both being under p0.001. Above all, the risk of moderate-severe illness was much less in the vaccinated category (16.7 vs 38.9, p=0.03) which provides a high degree of evidence that the vaccination reduces severe illness.

Table 4

Clinical Severity and Symptom Duration

Variable	Vaccinated (n=36)	Unvaccinated (n=36)	p-value
Symptomatic Infection, n (%)	23 (63.9%)	30 (83.3%)	0.06
Fever Duration (days), Mean ± SD	1.8 ± 0.9	3.4 ± 1.2	<0.001
Cough Duration (days), Mean ± SD	3.9 ± 1.5	6.2 ± 1.7	<0.001
Moderate–Severe Illness, n (%)	6 (16.7%)	14 (38.9%)	0.03

In all three circulating strains (H3N2 A, H1N1 B, and Reassortant C), the vaccinated group significantly had reduced mean duration of shedding (p<0.001 of H3N2 and H1N1 and p=0.002 of Reassortant C) between the vaccinated and unvaccinated groups. The advantageous influence of the vaccine on shedding of the virus is strong and seems to be applicable to various strains of viruses, which means that the effect is widespread. Although there is a slight variation in the shedding period of the variants of the two groups, the percentage change of one group compared to the other is extremely large in each case as a result of vaccination.

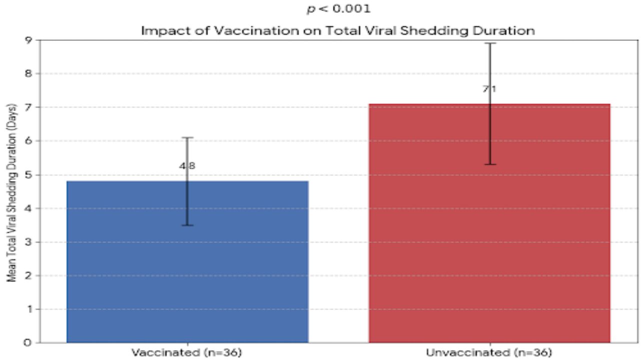
Table 5

Variant-Specific Shedding Differences

Variant	Vaccinated Mean Shedding (days)	Unvaccinated Mean Shedding (days)	p-value
H3N2 Variant A	5.1 ± 1.4	7.5 ± 1.9	<0.001
H1N1 Variant B	4.6 ± 1.2	6.8 ± 1.6	<0.001
Reassortant Variant C	5.3 ± 1.5	7.9 ± 2.0	0.002

Figure 1

Impact of Vaccination on Total Viral Shedding Duration



The most important finding is that the average total viral shedding time in the vaccinated group was 4.8 days; this is a 2.3 days less than the one seen in the unvaccinated group (p < 0.001). This finding is very strong in drawing the conclusion that vaccination reduces the infectious window.

DISCUSSION

The strongest conclusion of this research is the strong impact of influenza vaccination to inhibit viral replication and promote viral elimination. The vaccinated group showed much higher baseline and peak Ct values, which is associated with a lower initial and peak viral load which is consistent with the idea of immune-mediated attenuation in breakthrough infections. It is also indicated by the fact that the Rate of Viral Decline in vaccinated persons was significantly more rapid (2.8 Ct/day vs 1.9 Ct/day, p<0.001), and therefore it can be expected that the already established immune response, albeit too weak to prevent infection, actually limits the number and duration of viral growth [11-13].

It is obvious that the study provides the evidence of the positive impact of vaccination on the overall population health as it has a significantly shorter Total Shedding Duration (mean=4.8 days vs 7.1 days, p=0.001) and a much lower percentage of culture positive individuals on Day 5 (19.4 vs 61.1, p=0.001). One of the main proxies of infectivity is viral culture positivity, and this reduction is drastic, indicating that the vaccination does not only shorten the illness but also provides a narrowing of the possible transmission window. This result is essential, since the time spent sheddingeven at the time of year when vaccination effectiveness against infection is potentially suboptimal due to antigenic drift (as in the case of some H3N2 strains in 2021-2022) is an essential pay-off of vaccination [14, 15].

In line with research on influenza vaccination effect, this cohort showed that vaccination had a significant effect in alleviating clinical severity. The length of the period of fever and cough was much shorter in the group of vaccinated people ($p<0.001$ in each case), which speaks in favor of a less severe and less troublesome course of the disease [16, 17].

Most importantly, the percentage of Moderate-Severe Illness was reduced by more than half in the vaccinated group (16.7 vs 38.9, $p=0.03$), the key contribution of the vaccine: preventing severe outcomes and complications, i. e. hospitalization, which serves as the foundation of annual influenza vaccination campaigns. These results are consistent with meta-analyses done in the 2020-2022 that linked the probability of ICU admission and death to vaccination. [18].

One of the key findings is that the taming effect of the vaccine was sustained in all three circulating variants (H3N2 A, H1N1 B, and Reassortant C) and much shorter shedding periods were recorded in the vaccinated individuals in all the variant sub-groups (p 0.002). This implies some cross-protection/ T-cell mediated infection and immunity that offers wider and more effective protection against viral dynamics even against novel, drifted strains. Although the overall efficacy on the H3N2 in some seasons has been fluctuated regarding the occurrence of antigenic mismatch or egg-adaptation, the data presented here have provided a great indication that vaccination remains a quantitatively and statistically significant biological advantage in the control of the infection once it develops in whichever variant is in circulation [19, 20].

Strengths and Limitations

The primary strength of the study is that the nasopharyngeal swabs were collected meticulously and on a daily basis and both the quantitative RT-PCR (Ct values) and the qualitative viral culture were used to precisely determine the length of infectious shedding. The strong cohort demographic matching (Table 1) supports the conclusion that the differences are the most likely to be due to the vaccination status. The small size of the sample ($n=72$) is the limitation of the study as it limits the applicability of the results and the statistical power of the study to identify smaller variations in clinical outcomes. Moreover, the observational design, as required by the effectiveness studies in the real world, is not able to completely remove the residual confounding factors.

CONCLUSION

Influenza vaccine offers a significant level of protection against the severity and transmissibility of novel strains of influenza since there were significant and statistically significant differences in the dynamics of the virus and clinical results. Vaccination causes greatly reduced viral loads, accelerated viral clearance, and a greatly reduced infectious viral shedding. These effects are manifested in a less severe disease course, which is shorter periods of fever and cough, as well as a reduced risk of moderate-severe illness significantly. Such results reinforce the importance of annual influenza vaccination as an effective tool of influencing the relationship between disease burden of individual persons and the risk of community-wide transmission of the virus even in those years when the number of variants circulated is high.

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