

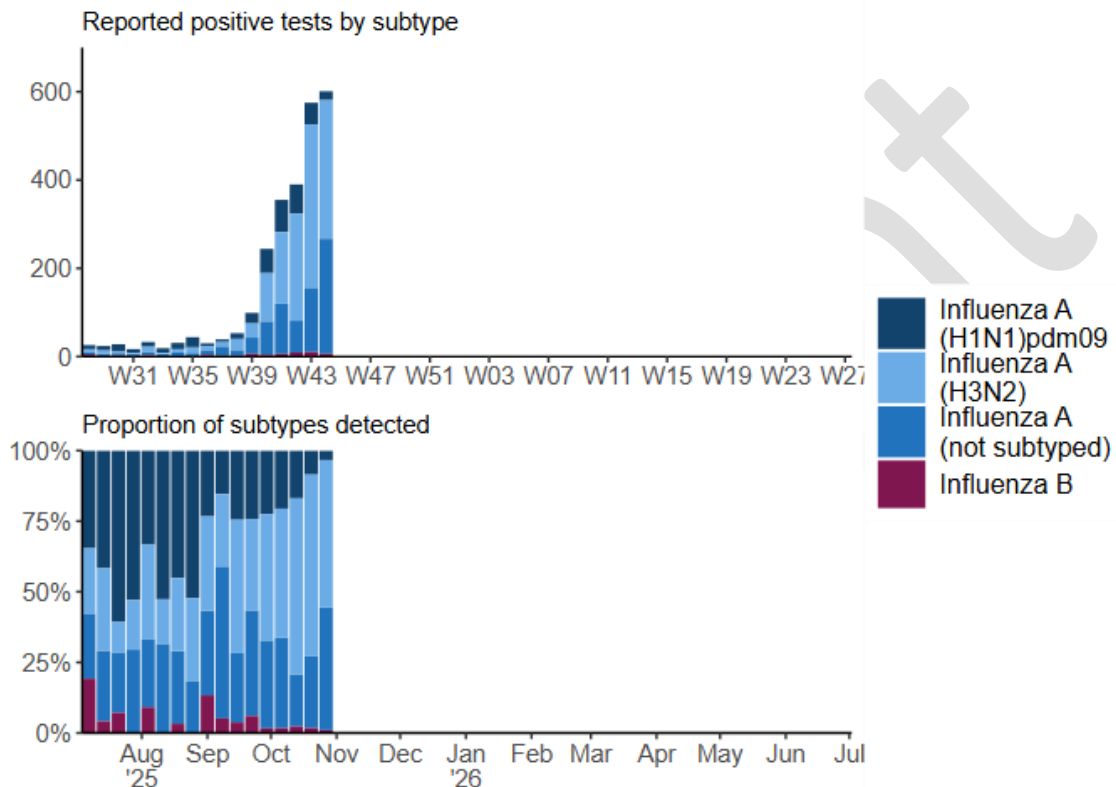
Supplementary appendix

Early influenza virus characterisation and vaccine effectiveness in England in autumn 2025, a period dominated by influenza A(H3N2) subclade K

Pre-print

Supplementary Figure 1.

Surveillance data from the Respiratory Datamart surveillance system in England¹ (covering the national reference laboratory, regional public health laboratories and sentinel hospital laboratories), showing the number and proportion of influenza tests by subtype. Data is taken from the UK Health Security Agency National flu and COVID-19 surveillance report: 6 November 2025 (week 45)².



¹ <https://www.gov.uk/government/publications/sources-of-surveillance-data-for-influenza-covid-19-and-other-respiratory-viruses/data-quality-report-national-flu-and-covid-19-surveillance-report#laboratory-surveillance>

² <https://www.gov.uk/government/statistics/national-flu-and-covid-19-surveillance-reports-2025-to-2026-season/national-flu-and-covid-19-surveillance-report-6-november-2025-week-45>

Supplementary genetic and antigenic characterisation methods

Influenza virus genetic and antigenic characterisation in England is undertaken at the Respiratory Virus Unit (RVU) national reference laboratory and is comprised of 2 main data sources; primary care and secondary care virological surveillance.

Primary care sentinel surveillance is undertaken via a subset of Oxford-Royal College of General Practitioners Research and Surveillance Centre (RCGP-RSC) network practices (around 300) that participate in weekly virology surveillance. Practices collect nasopharyngeal samples from patients presenting to their general practitioner (GP) with symptoms of any acute respiratory infection or influenza-like illness with an onset date within the last 10 days. Samples are transported to the RVU reference laboratory for primary testing.

Secondary care surveillance occurs via UKHSA regional Clinical Network Laboratories and some NHS laboratories that refer influenza positive samples to RVU for further characterization.

Influenza detection by RT-PCR and whole genome sequencing

Primary care and referred secondary care samples were tested using in-house developed multiplexed real-time RT-PCR assays to determine influenza type and subtype. A subset of samples underwent genetic characterization by whole genome sequencing using an in-house workflow as previously described³. Briefly, following RNA extraction, one-step RT-PCR was performed using influenza-specific primers⁴. Sequencing libraries were prepared for sequencing using the DNA Prep library preparation kit (Illumina) from generated amplicons and sequenced using short-read, Illumina sequencing technology. Reads were mapped with BWA v0.7.5 and converted to BAM files using SAMTools (1.1.2). Variants were called using QuasiBAM, an in-house developed script was used to analyse the consensus sequence for signature amino acid changes and phylogenetic clustering to determine the genetic clade or subclade.

Virus culture and antigenic characterisation

After sequencing, a representative subset of influenza viruses from clinical samples as well as influenza viruses with novel or unusual changes identified by genomic surveillance are selected for virus culture and antigenic characterisation by haemagglutination inhibition (HAI) assay. HAI was performed using standard methods⁵.

³ Goldhill DH, Langat P, Xie H, et al. Determining the Mutation Bias of Favipiravir in Influenza Virus Using Next-Generation Sequencing. *J Virol*. 2019;93(2):e01217-18. Published 2019 Jan 4. doi:10.1128/JVI.01217-18

⁴ Zhou B, Donnelly ME, Scholes DT, George KS, Hatta M, Kawaoka Y, Wentworth DE. 2009. Single-reaction genomic amplification accelerates sequencing and vaccine production for classical and Swine origin human influenza A viruses. *J Virol* 83:10309–10313. doi: 10.1128/JVI.01109-09.

⁵ WHO 2011. WHO global influenza surveillance network: manual for the laboratory diagnosis and virological surveillance of influenza. <https://www.who.int/publications/i/item/manual-for-the-laboratory-diagnosis-and-virological-surveillance-of-influenza>

Supplementary vaccine effectiveness methods

Data sources

Influenza testing data

Laboratory testing data was extracted on 7 November 2025. Two sources of laboratory PCR testing data were used to identify influenza positive cases and influenza negative controls in England. First, the Respiratory Datamart, a sentinel laboratory surveillance system, with 9 participating laboratories in the 2025 to 2026 influenza season, and secondly, the Second-Generation Surveillance System (SGSS), which records laboratory outcomes across England. Testing from the Respiratory Datamart are largely a subset of those in SGSS, with additional influenza A subtype information available for some laboratories. Datamart and SGSS were also used to identify the influenza samples which had also been tested for COVID-19.

Immunisation Information System (IIS)

The testing data was linked to the UKHSA IIS (a national vaccine register containing vaccine histories and demographic information on the whole population of England registered with a GP), using combinations of the unique individual NHS number, date of birth, surname, first name, and postcode using deterministic linkage. The IIS was accessed for dates of influenza vaccination and vaccine type, demographic data including sex, date of birth, ethnicity, index of multiple deprivation (IMD) quintile (small area measures of relative deprivation based on postcode) and NHS region. Clinical risk group status (those identified as being eligible for an influenza vaccine by NHS Cohorting as a Service (CaaS) was also extracted.

Emergency Care Data Set (ECDS)

Testing and vaccination data was linked to England's national Emergency Care Data Set (ECDS) to identify ED attendances and hospital admissions ECDS is the national dataset for urgent and emergency care in England. It includes hospital admissions through emergency department but not elective admissions. Testing data was linked to ECDS using NHS number and date of birth to identify ED attendances and hospital admissions within -2 to 14 days of the test. Admissions due to an injury were excluded. Admissions with the reason for attending emergency care being a SNOMED CT (Systematized Nomenclature of Medicine-Clinical Terms) coded acute respiratory illness were flagged. The study period for the ECDS analysis was 29 September to 02 November 2025.

Secondary Uses Service

A sensitivity analysis was also run using the Secondary Uses Service (SUS), the national electronic database of hospital admissions that provides timely updates of ICD-10 codes for completed hospital stays for all NHS hospitals in England. Testing data was linked to SUS using NHS number and date of birth. SUS was used to identify hospitalisations within 14 days of a respiratory swab, or where a swab was taken up to 2 days after admission. The study period for the SUS analysis was 29 September to 26 October 2025, to allow for more data lags than the ECDS dataset.

Exclusions

Tests from individuals aged 2 years and older (on 31st August 2025) and resident in England were included. Tests without a sample date were excluded, as were tests where the influenza status was not fully known (that is controls required both influenza A and B negative results). Testing data was first de-duplicated such that no more than one test per person per 28-day period was retained, and a new positive test was kept over a negative test so that any positive test around the time of a respiratory hospital admission defined a case. We then further restricted to inclusion of the first of each influenza A(H1N1)pdm09, A(H3N2) and B positive test, and where influenza A was not subtyped, we ensured positive tests were at least 6 weeks apart. We further excluded tests with no linkage to IIS, within 0 to

13 days of vaccination, plus adults with a record of receiving LAIV and children that received a recombinant or adjuvanted vaccine. Given the association between COVID-19 and influenza vaccination and its impact on the vaccination status of non-cases, SARS-CoV-2 positive controls were removed.

Covariates and adjustment

Vaccination status was the primary exposure variable of interest in all analyses. Additionally, all analyses included adjustment for week of test date, age group (2 to 3, 4 to 6, 7 to 10, 11 to 15, 16 to 17, 18 to 34, 35 to 49, 50 to 64, 65 to 74, 75 to 84, 85+), region and clinical risk group status (encoded as a categorical variable with a level for all conditions other than immunosuppression and a level for immunosuppression (as defined in the Green book). We assessed sex, ethnicity and IMD as potential confounders but did not include these in the final model as they did not change the vaccine effect by more than 1% so their inclusion was not deemed necessary.

Statistical methods

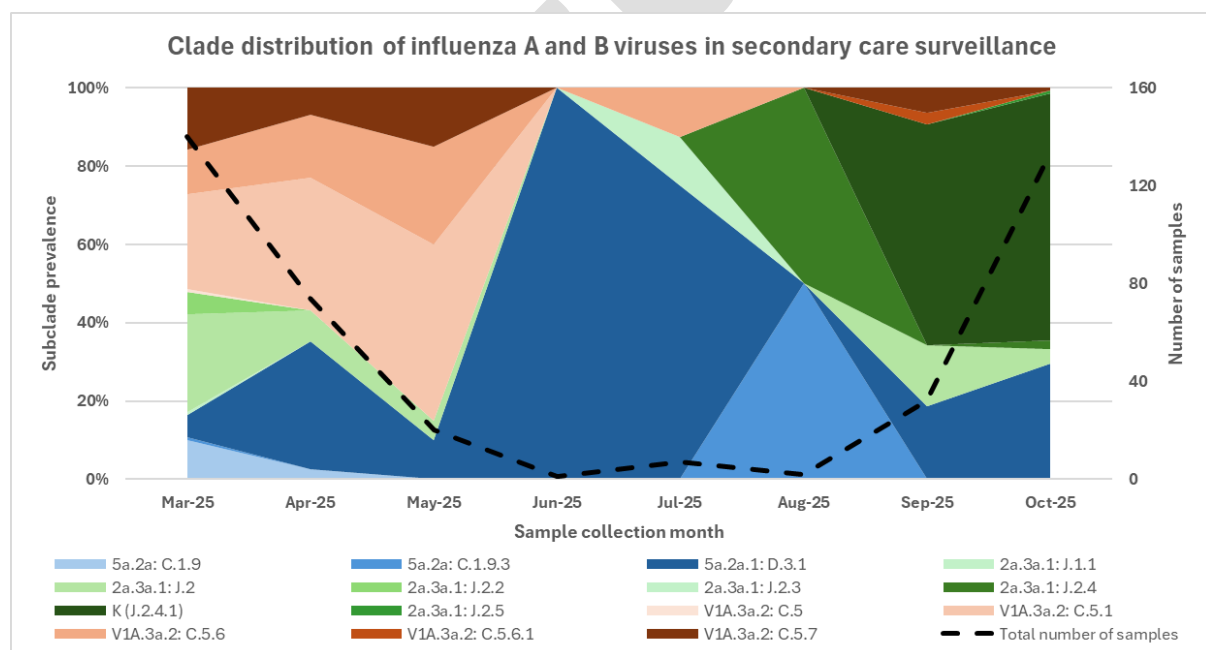
Multivariable logistic regression was used with the test result as the outcome, vaccination status as the primary exposure variable of interest and with confounder adjustment as described above. VE was calculated as 1-odds ratio and given as a percentage. To compare estimates, statistical significance was concluded where 95% confidence intervals (CIs) did not overlap. Sensitivity analyses were conducted restricting the ED attendances and hospital admissions from ECDS to those coded as an acute respiratory attendance, and estimating VE against hospitalisation using the SUS dataset.

Supplementary Figure 2.

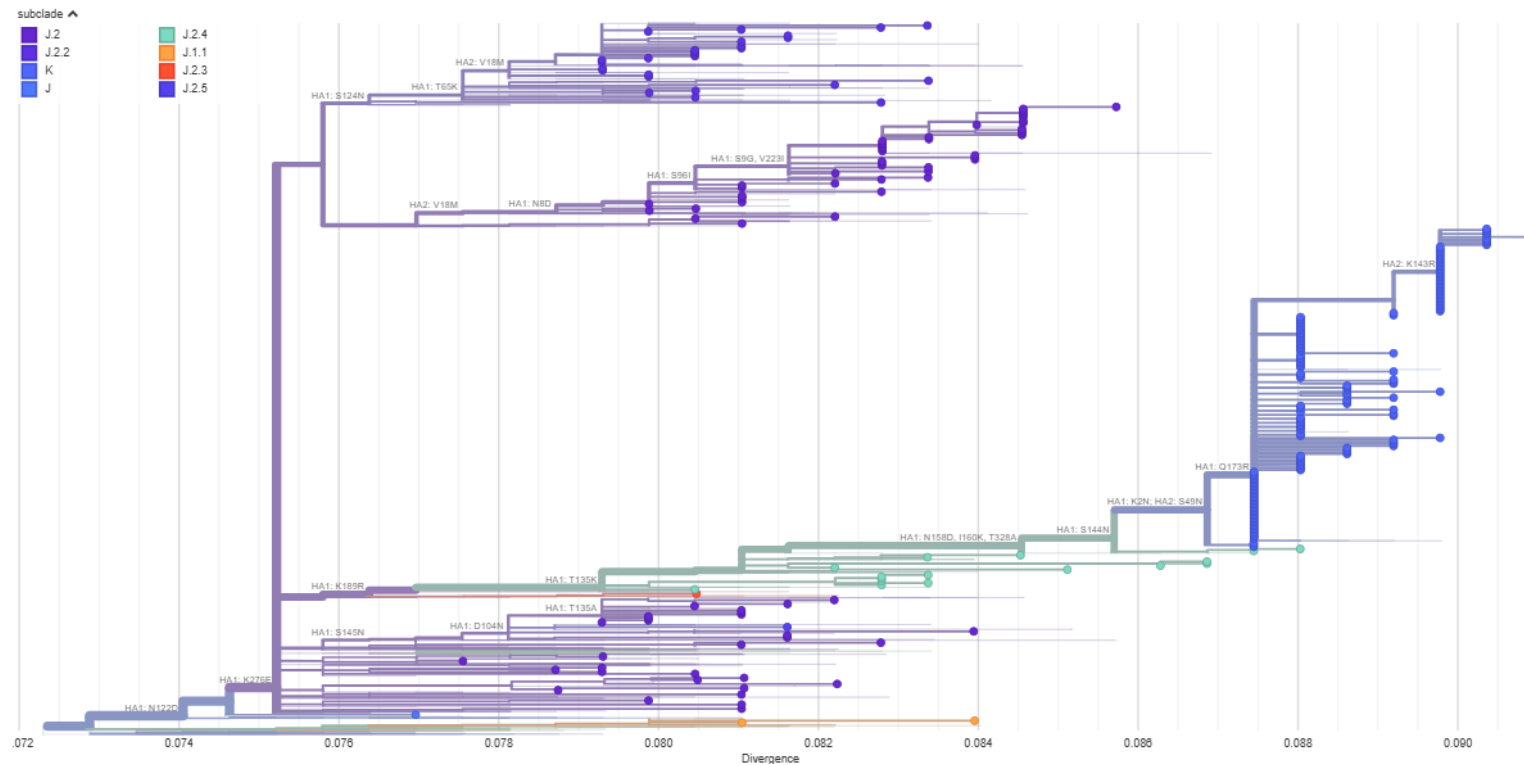
Supplementary Figure 2a Throughout early summer (after week 20), Influenza A(H1N1)pdm09 dominated the circulating viruses (64% of all influenza detections) – A(H1N1)pdm09 detections belonged to genetic clade 5a.2a.1, subclade D.3.1. Antigenic analysis of viruses showed that the majority of these (97%) were well inhibited by post-infection ferret antisera raised against egg-propagated A/Victoria/4897/2022-like and cell culture-propagated A/Wisconsin/67/2022-like viruses, representing the vaccine viruses for the 2025 to 2026 Northern Hemisphere influenza seasons.

During early summer, influenza B and H3N2 viruses were detected at equal levels (18% each). All genetically analysed influenza B viruses from this period were in the V1A.3a.2 clade of the B/Victoria lineage and reacted well with post-infection ferret antisera raised to tissue-culture grown virus B/Austria/1359417/2021 representative of the northern Hemisphere vaccine strain for 2024 to 2025. Most H3N2 viruses from this period genetically belonged to the clade 2a.3a.1. (mostly in the J.2 subclade) and were well inhibited by post-infection ferret antisera raised against tissue culture derived A/DistrictofColumbia/27/2023 and post-infection ferret antisera raised against egg-propagated A/Croatia/10136RV/2023, the Southern hemisphere 2025 vaccine strain components.

However, sporadic detections of samples with amino acid substitution distinguishing these as subclade J.2.3, J.2.4 and J.2.5. were also observed. From August, an increasing proportion of influenza detections was influenza A subtype H3N2 – amongst which proportion of subclade K viruses increased noticeably from week 36. This change in composition of H3N2 viruses is accompanied with a trend towards reduced reactivity over time, while most viruses from the J.2 and J.2.2 subclades continued to react well (titres within 4-fold of the homologous titre) with post-infection ferret antisera raised against the NH 2025 to 2026 vaccine strains: egg-propagated A/Croatia/10136RV/2023 (14 out of 25; 56%) and cell-propagated A/District of Columbia/27/2023 (25 out of 25 100%).



Supplementary Figure 2b. Phylogenetic analysis of 278 influenza A(H3N2) full-length segment 4 (haemagglutinin) sequences collected between week 10 2025 and week 43 2025 with root EPI1857216 generated using NextClade^{6,7}. UKHSA isolates are shown as balls at the tip among a subset of international strains as thin branches. Different subclades are shown in different colours. The X-axis represents genetic divergence.

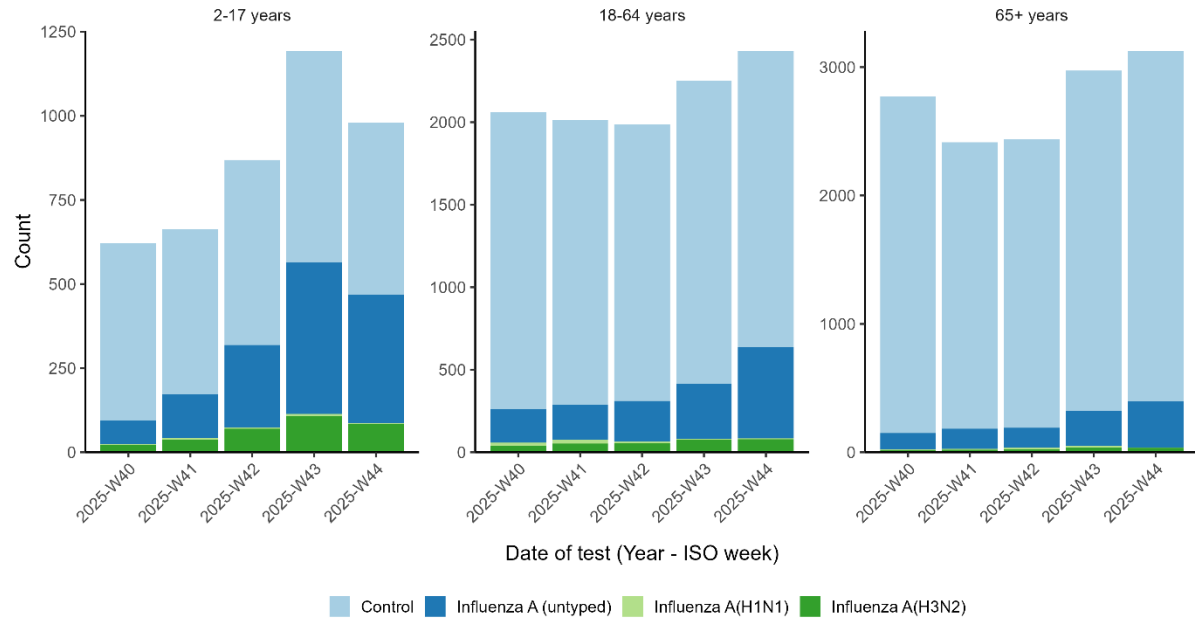


⁶ <https://clades.nextstrain.org>

⁷ Aksamentov, I., Roemer, C., Hodcroft, E. B., & Neher, R. A., (2021). Nextclade: clade assignment, mutation calling and quality control for viral genomes. Journal of Open Source Software, 6(67), 3773, <https://doi.org/10.21105/joss.03773>

Supplementary Figure 3.

The distribution of cases and controls over time during the study, amongst children aged 2 to 17 years, adults aged 18 to 64 years and adults aged 65 years and older.



Supplementary Table 1

Descriptive characteristics of children aged 2 to 17 years included in the analysis estimating vaccine effectiveness against ED attendance.

Characteristic	Controls	Influenza A	Influenza A(H1N1)	Influenza A(H3N2)
	N = 2,707 ¹	N = 1,618 ¹	N = 22 ¹	N = 321 ¹
Vaccination Status				
Unvaccinated	2,372 (88%)	1,558 (96%)	18 (82%)	311 (97%)
Vaccinated	335 (12%)	60 (3.7%)	4 (18%)	10 (3.1%)
Age (years)				
2-3	955 (35%)	393 (24%)	10 (45%)	78 (24%)
4-6	643 (24%)	447 (28%)	6 (27%)	78 (24%)
7-10	459 (17%)	264 (16%)	2 (9.1%)	47 (15%)
11-15	440 (16%)	404 (25%)	4 (18%)	98 (31%)
16-17	210 (7.8%)	110 (6.8%)	0 (0%)	20 (6.2%)
Region				
East Midlands	223 (8.2%)	183 (11%)		4 (1.2%)
East of England	182 (6.7%)	47 (2.9%)	3 (14%)	7 (2.2%)
London	304 (11%)	190 (12%)		14 (4.4%)
North East	353 (13%)	128 (7.9%)		1 (0.3%)
North West	641 (24%)	604 (37%)	6 (27%)	252 (79%)
South East	281 (10%)	111 (6.9%)		2 (0.6%)
South West	348 (13%)	62 (3.8%)	8 (36%)	13 (4.0%)
West Midlands	215 (7.9%)	179 (11%)	1 (4.5%)	22 (6.9%)
Yorkshire and Humber	160 (5.9%)	114 (7.0%)	4 (18%)	6 (1.9%)
Clinical Risk Status				
No risk	2,101 (78%)	1,335 (83%)	17 (77%)	256 (80%)
At risk; not immunosuppressed	468 (17%)	226 (14%)	2 (9.1%)	47 (15%)
At risk; immunosuppressed	138 (5.1%)	57 (3.5%)	3 (14%)	18 (5.6%)
¹ n (%)				

Supplementary Table 2

Descriptive characteristics of adults aged 18 to 64 years included in the analysis estimating vaccine effectiveness against ED attendance.

Characteristic	Controls	Influenza A	Influenza A(H1N1)	Influenza A(H3N2)
	N = 8,831 ¹	N = 1,913 ¹	N = 61 ¹	N = 304 ¹
Vaccination Status				
Unvaccinated	8,353 (95%)	1,835 (96%)	60 (98%)	298 (98%)
Vaccinated	478 (5.4%)	78 (4.1%)	1 (1.6%)	6 (2.0%)
Age (years)				
18-34	2,416 (27%)	976 (51%)	15 (25%)	175 (58%)
35-49	2,481 (28%)	436 (23%)	11 (18%)	75 (25%)
50-64	3,934 (45%)	501 (26%)	35 (57%)	54 (18%)
Region				
East Midlands	432 (4.9%)	203 (11%)	1 (1.6%)	7 (2.3%)
East of England	668 (7.6%)	89 (4.7%)	5 (8.2%)	20 (6.6%)
London	1,071 (12%)	258 (13%)	2 (3.3%)	25 (8.2%)
North East	1,147 (13%)	119 (6.2%)		2 (0.7%)
North West	1,677 (19%)	542 (28%)	14 (23%)	178 (59%)
South East	829 (9.4%)	111 (5.8%)	2 (3.3%)	2 (0.7%)
South West	1,212 (14%)	129 (6.7%)	21 (34%)	10 (3.3%)
West Midlands	1,064 (12%)	208 (11%)	10 (16%)	51 (17%)
Yorkshire and Humber	731 (8.3%)	254 (13%)	6 (9.8%)	9 (3.0%)
Clinical Risk Status				
No risk	4,082 (46%)	1,118 (58%)	24 (39%)	199 (65%)
At risk; not immunosuppressed	3,860 (44%)	665 (35%)	33 (54%)	85 (28%)
At risk; immunosuppressed	889 (10%)	130 (6.8%)	4 (6.6%)	20 (6.6%)
¹ n (%)				

Supplementary Table 3

Descriptive characteristics of adults aged 65 years and older included in the analysis estimating vaccine effectiveness against ED attendance.

Characteristic	Controls	Influenza A	Influenza A(H1N1)	Influenza A(H3N2)
	N = 12,479 [†]	N = 1,241 [†]	N = 50 [†]	N = 130 [†]
Vaccination Status				
Unvaccinated	10,442 (84%)	1,048 (84%)	48 (96%)	112 (86%)
Vaccinated	2,037 (16%)	193 (16%)	2 (4.0%)	18 (14%)
Age (years)				
65-74	3,882 (31%)	448 (36%)	12 (24%)	51 (39%)
75-84	4,964 (40%)	483 (39%)	24 (48%)	41 (32%)
85+	3,633 (29%)	310 (25%)	14 (28%)	38 (29%)
Region				
East Midlands	546 (4.4%)	128 (10%)	1 (2.0%)	3 (2.3%)
East of England	999 (8.0%)	52 (4.2%)	2 (4.0%)	5 (3.8%)
London	1,040 (8.3%)	173 (14%)		17 (13%)
North East	1,654 (13%)	57 (4.6%)		
North West	2,073 (17%)	332 (27%)	12 (24%)	53 (41%)
South East	1,267 (10%)	75 (6.0%)	1 (2.0%)	2 (1.5%)
South West	2,029 (16%)	114 (9.2%)	22 (44%)	11 (8.5%)
West Midlands	1,773 (14%)	160 (13%)	12 (24%)	39 (30%)
Yorkshire and Humber	1,098 (8.8%)	150 (12%)		
Clinical Risk Status				
No risk	2,004 (16%)	212 (17%)	10 (20%)	18 (14%)
At risk; not immunosuppressed	8,855 (71%)	886 (71%)	32 (64%)	91 (70%)
At risk; immunosuppressed	1,620 (13%)	143 (12%)	8 (16%)	21 (16%)
[†] n (%)				

Supplementary Table 4

Descriptive characteristics of children aged 2 to 17 years included in the analysis estimating vaccine effectiveness against hospital admission.

Characteristic	Controls	Influenza A	Influenza A(H1N1)	Influenza A(H3N2)
	N = 2,237 ¹	N = 962 ¹	N = 20 ¹	N = 298 ¹
Vaccination Status				
Unvaccinated	1,956 (87%)	924 (96%)	17 (85%)	288 (97%)
Vaccinated	281 (13%)	38 (4.0%)	3 (15%)	10 (3.4%)
Age (years)				
2-3	789 (35%)	240 (25%)	9 (45%)	70 (23%)
4-6	525 (23%)	259 (27%)	5 (25%)	70 (23%)
7-10	398 (18%)	151 (16%)	2 (10%)	44 (15%)
11-15	364 (16%)	248 (26%)	4 (20%)	94 (32%)
16-17	161 (7.2%)	64 (6.7%)	0 (0%)	20 (6.7%)
Region				
East Midlands	150 (6.7%)	83 (8.6%)		4 (1.3%)
East of England	176 (7.9%)	41 (4.3%)	3 (15%)	7 (2.3%)
London	242 (11%)	90 (9.4%)		6 (2.0%)
North East	271 (12%)	78 (8.1%)		1 (0.3%)
North West	590 (26%)	397 (41%)	6 (30%)	240 (81%)
South East	219 (9.8%)	70 (7.3%)		1 (0.3%)
South West	306 (14%)	41 (4.3%)	7 (35%)	12 (4.0%)
West Midlands	169 (7.6%)	108 (11%)	1 (5.0%)	22 (7.4%)
Yorkshire and Humber	114 (5.1%)	54 (5.6%)	3 (15%)	5 (1.7%)
Clinical Risk Status				
No risk	1,704 (76%)	752 (78%)	15 (75%)	233 (78%)
At risk; not immunosuppressed	407 (18%)	162 (17%)	2 (10%)	47 (16%)
At risk; immunosuppressed	126 (5.6%)	48 (5.0%)	3 (15%)	18 (6.0%)
¹ n (%)				

Supplementary Table 5

Descriptive characteristics of adults aged 18 to 64 years included in the analysis estimating vaccine effectiveness against hospital admission.

Characteristic	Controls	Influenza A	Influenza A(H1N1)	Influenza A(H3N2)
	N = 7,221 ¹	N = 1,331 ¹	N = 57 ¹	N = 293 ¹
Vaccination Status				
Unvaccinated	6,831 (95%)	1,273 (96%)	56 (98%)	288 (98%)
Vaccinated	390 (5.4%)	58 (4.4%)	1 (1.8%)	5 (1.7%)
Age (years)				
18-34	1,878 (26%)	630 (47%)	13 (23%)	172 (59%)
35-49	1,982 (27%)	302 (23%)	9 (16%)	70 (24%)
50-64	3,361 (47%)	399 (30%)	35 (61%)	51 (17%)
Region				
East Midlands	344 (4.8%)	165 (12%)	1 (1.8%)	7 (2.4%)
East of England	625 (8.7%)	73 (5.5%)	5 (8.8%)	20 (6.8%)
London	796 (11%)	134 (10%)		14 (4.8%)
North East	893 (12%)	81 (6.1%)		2 (0.7%)
North West	1,349 (19%)	377 (28%)	14 (25%)	178 (61%)
South East	718 (9.9%)	84 (6.3%)	2 (3.5%)	2 (0.7%)
South West	965 (13%)	96 (7.2%)	21 (37%)	10 (3.4%)
West Midlands	971 (13%)	162 (12%)	10 (18%)	51 (17%)
Yorkshire and Humber	560 (7.8%)	159 (12%)	4 (7.0%)	9 (3.1%)
Clinical Risk Status				
No risk	3,147 (44%)	698 (52%)	21 (37%)	191 (65%)
At risk; not immunosuppressed	3,312 (46%)	530 (40%)	32 (56%)	82 (28%)
At risk; immunosuppressed	762 (11%)	103 (7.7%)	4 (7.0%)	20 (6.8%)
¹ n (%)				

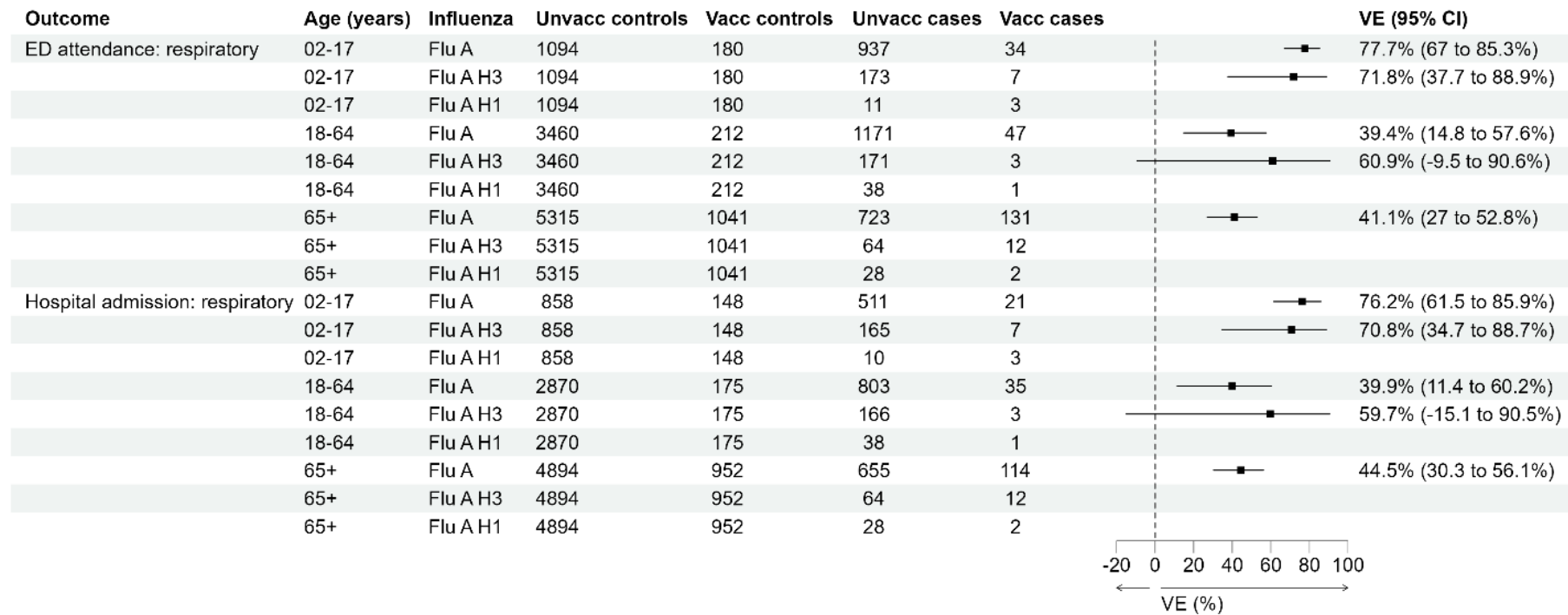
Supplementary Table 6

Descriptive characteristics of adults aged 65 years and older included in the analysis estimating vaccine effectiveness against hospital admission.

Characteristic	Controls	Influenza A	Influenza A(H1N1)	Influenza A(H3N2)
	N = 11,200 [†]	N = 1,103 [†]	N = 50 [†]	N = 129 [†]
Vaccination Status				
Unvaccinated	9,404 (84%)	942 (85%)	48 (96%)	111 (86%)
Vaccinated	1,796 (16%)	161 (15%)	2 (4.0%)	18 (14%)
Age (years)				
65-74	3,481 (31%)	386 (35%)	12 (24%)	51 (40%)
75-84	4,442 (40%)	434 (39%)	24 (48%)	41 (32%)
85+	3,277 (29%)	283 (26%)	14 (28%)	37 (29%)
Region				
East Midlands	495 (4.4%)	123 (11%)	1 (2.0%)	3 (2.3%)
East of England	980 (8.8%)	48 (4.4%)	2 (4.0%)	5 (3.9%)
London	961 (8.6%)	151 (14%)		16 (12%)
North East	1,144 (10%)	33 (3.0%)		
North West	1,843 (16%)	279 (25%)	12 (24%)	53 (41%)
South East	1,176 (11%)	72 (6.5%)	1 (2.0%)	2 (1.6%)
South West	1,896 (17%)	106 (9.6%)	22 (44%)	11 (8.5%)
West Midlands	1,700 (15%)	156 (14%)	12 (24%)	39 (30%)
Yorkshire and Humber	1,005 (9.0%)	135 (12%)		
Clinical Risk Status				
No risk	1,781 (16%)	182 (17%)	10 (20%)	18 (14%)
At risk; not immunosuppressed	7,966 (71%)	786 (71%)	32 (64%)	90 (70%)
At risk; immunosuppressed	1,453 (13%)	135 (12%)	8 (16%)	21 (16%)
[†] n (%)				

Supplementary Figure 4.

Sensitivity analysis estimating vaccine effectiveness by influenza type and subtype, restricting to respiratory coded ED attendances and hospital admissions.



Supplementary Figure 5.

Sensitivity analysis estimating vaccine effectiveness against hospital admissions using the Secondary Use Services (SUS) dataset.

