Web Extra material: Influenza virus infection history drives and shapes antibody responses to influenza vaccination

Section 1: Study design and population

A prospective, observational study was commenced in November 2016 to assess influenza vaccine immunogenicity among a purposefully selected subset of adult participants of the Ha Nam cohort. This cohort was established in Ha Nam, Viet Nam in December 2007, and comprised 270 households, which initially included 945 household members. Participants have been intensively monitored for influenza infection by active influenza-like-illness (ILI) surveillance, combined with (bi)annual collection of blood samples, at times spanning transmission peaks, for influenza virus serology. Infection was defined as having RT-PCR-confirmed ILI or a four-fold or greater rise in antibody titres (seroconversion) against a circulating strain.

Consent for continued participation in the cohort was obtained in 2007, 2009, 2013, and most recently in July 2016 when 765 participants from 201 households remained, including 556 aged at least 18 years. Three hundred and eighty-two adults also consented to participate in sub-studies that required additional blood samples and volumes to assess immune responses to vaccination or infection. Of these, 159 had provided blood samples at all 12 time points since December 2007, and were present during all influenza transmission periods, such that their 9 Y infection histories could be documented. We selected all 28 who lacked a detectable A(H3N2) infection, then purposefully selected 72 from the group with at least one A(H3N2) virus infection to have a similar sex and age distribution.

Trivalent inactivated influenza vaccine (TIV, Vaxigrip, Sanofi Pasteur) was administered to the 100 selected participants in November 2016. The virus strains included in this vaccine were A/Hong Kong/4801/2014 (H3N2)-like, A/California/7/2009 (H1N1)pdm09-like, and B/Brisbane/60/2008-like. Venous blood samples were collected into serum and sodium heparin tubes pre-vaccination, and post-vaccination on days 4, 7, 14, 21, and 280 (**Figure S1**). Blood samples were also collected 7 and 21 days after confirmed influenza illness, detected via continued active ILI surveillance.

The vaccine study was approved by the University of Melbourne Health Sciences Human Ethics Committee (HREC # 1646470), the Institutional Review Board of the National Institute of Hygiene and Epidemiology, Viet Nam (NIHE IRB-VN01057 – 08/2016) and the Oxford Tropical Research Ethics Committee (OXTREC # 30-16). The Ha Nam Cohort study was approved by the National Institute of Hygiene and Epidemiology, Viet Nam (NIHE IRB-VN01057 – 08/2016) and by the Oxford Tropical Research Ethics Committee (OXTREC # 019-07). Participants provided informed consent, conducted in Vietnamese.

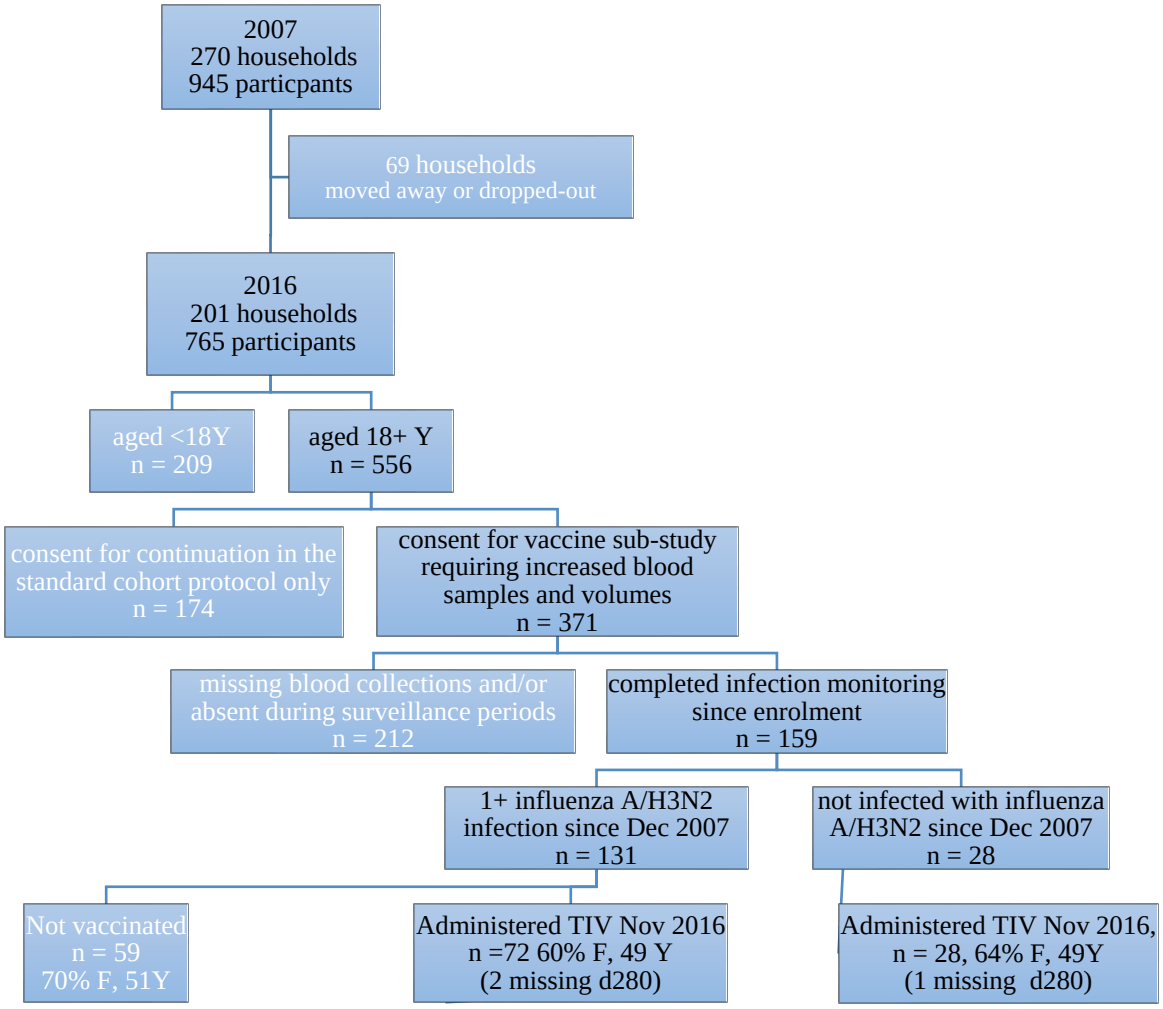

Figure S1. Study flow diagram. 100 adult participants of the Ha Nam household influenza cohort were selected as depicted in the Flowchart above. Participants included and excluded at each selection stage are indicated by black and white text boxes, respectively.

Section 2: Laboratory protocols

Hemagglutination inhibition (HI) assays

HI assays were performed according to WHO Global Influenza Surveillance Network protocols [WHO Global Influenza Surveillance Network Manual for the laboratory diagnosis and virological surveillance of influenza] with the exception that wells contained 25 µL each of diluted sera, virus and 1% Guinea Pig erythrocytes (0.33% final) instead of 50 µL of 0.75% erythrocytes (0.375% final). Viruses were propagated in Madin Darby Canine Kidney (MDCK) cells, human 2,6-sialtransferase transfected MDCK (SIAT) cells or eggs (**Table S1**). Sera were tested over two-fold serial dilutions from 1:10 through 1:10240. Each individual's complete set of sera were tested against all viruses using the same erythrocyte preparation, Quality control viruses and sera were run with each new batch of samples/erythrocytes and were accepted if HA and HI titres were within 2-fold of initial values. HI titres were read using an automated reader (CypherOne, InDevR). Reading settings (calibration factor = 165 and transition point = 782) were initially optimized by comparison with manual titre reads (**Figure S2**), then applied to all plates.

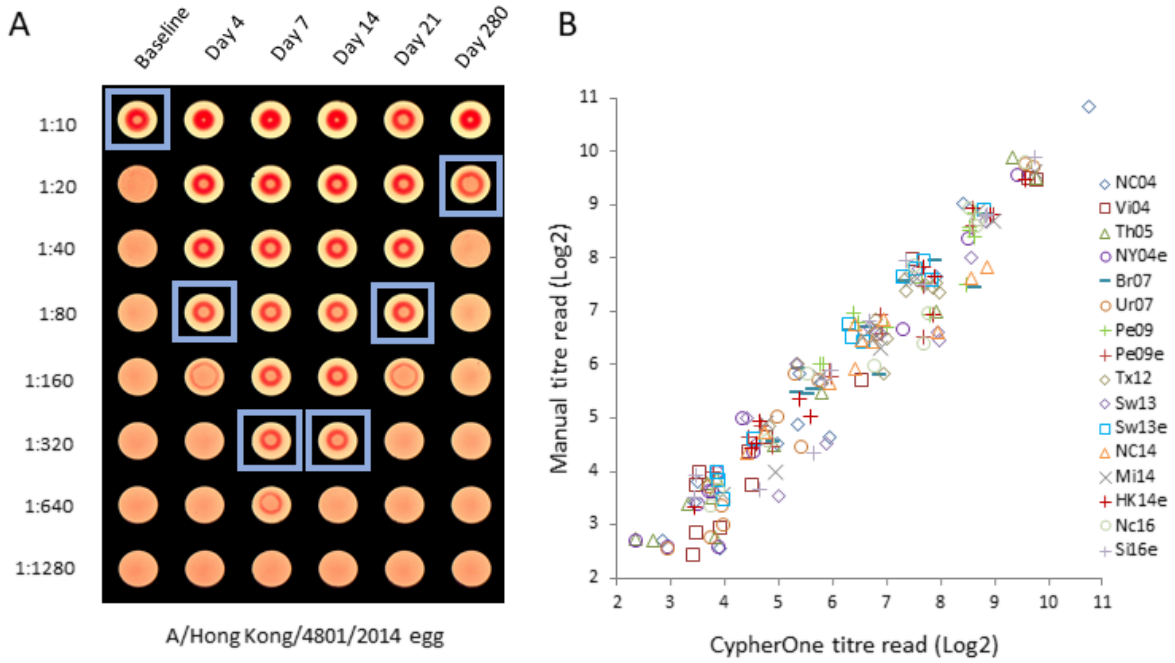

Figure S2. HI assay reading. (A) example of images captured and titres determined using the CypherOne reader, showing sera collected at all time points from one participant against HK14e. (B) Comparison of titres read via CypherOne versus manually for a range of human and ferret sera against the indicated viruses.

Genetic and antigenic characterization of viruses

HA and NA genes of viruses used for serology (**Table S1**) and/or from swabs of Ha Nam Cohort participants (isolates or clinical specimens) were sequenced via Sanger sequencing and aligned using the Multiple Alignment using Fast Fourier Transform (MAFFT) algorithm in MegAlign Pro 13 (DNASTAR Lasergene 13). Phylogenetic trees were edited in FigTree Version 1.4.4 (2006-2018, Andrew Rambaut, Institute of Evolutionary Biology, University of Edinburgh. http://tree.bio.ed.ac.uk/).

Viruses circulating since 2007 were tested in HI using a panel of antisera. Antisera were raised by infection of ferrets with viruses that were homologous or from the same genetic clade (Table S2). Antigenic cartography software was used to generate a two-dimensional map of virus antigenic distances from the matrix of two-way titres of each sera against each virus.

Table S1. A(H3N2) viruses used for serology

Year	Designation	Passage [a]	NA aa sequence 147-151 (X mix)	HA Titre [b,c] T	HA Titre [b,c] GP	HA Titre [b,c] GP^{OST}	HI Titre [c,d] PBS	HI Titre [c,d] OST	sera n	*p*
1968	A/Bilthoven/16190/68	X,MDCK3	DTIHD	16	32	64	47	42	45	·233
1972	A/Bilthoven/21793/72	MDCK3	DTIHD	64	128	128	**44**	31	20	·002
1975	A/Bilthoven/1761/76	MDCK3	DTIHD	32	64	64	**44**	33	29	·005
1977	A/Bilthoven/2271/76	X,MDCK3	DTIHD	32	128	128	**67**	51	20	·002
1979	A/Netherlands/233/82	tMK1,MDCK4	DTIHD	16	32	32	36	33	44	·199
1982	A/Philippines/2/82	MDCKX,2	DTIHD	16	16	32	62	44	20	·163
1987	A/Netherlands/620/89	X,tMK1,MDCK3	DTVHD	32	32	64	59	58	31	·768
1989	A/Netherlands/823/92	X,MDCK3	DTVHD	32	32	32	56	52	20	·606
1993	A/Netherlands/179/93	X,MDCK3	DTVHD	32	32	32	**74**	57	43	·000
1995	A/Netherlands/178/95	293T,MDCK4	DTVHD	16	32	32	**88**	61	15	·001
1997	A/Tasmania/1/97	MDCK7	DTVHD	64	32	32	40	34	20	·259
1999	A/Netherlands/301/99	MDCK5	DTVHD	16	16	32	140	120	31	·090
1999	A/Townsville/2/99	MDCK2, SIAT1^{p}	DTVHX(D|G)	*16*	128	64	57	**293**	8	·005
2002	A/Philippines/472/02	MDCK6^{p}	DTVHX(D|N)	4	16	8	58	**101**	20	·009
2002	A/Fujian/411/02	X,MDCK9, SIAT1	DTVHD	8	32	24	113	109	43	·594
2004	A/Victoria/511/04	MDCKx,2^{p}	DTVHX(D|N|S|G)	32	64	16	45	**69**	18	·011
2005	A/Thailand/409/05	P2,MDCK2^{p}	DTVHX(D|G)	32	64	32	34	32	18	·331
2007	A/Brisbane/10/07	MDCKX,5,SIAT1^{p}	DxVRX(k|i)(N|E|K)	24	128	32	75	78	18	·298
2008	A/HaNam/EL134/08	MDCK4, SIAT1 ^{p}	NxVRD(T|K)	16	32	16	73	**135**	8	.064
2009	A/HaNam/EL201/09	MDCKX,3,SIAT1	NTVRD	na	8	8	80	70	5	.621
2009	A/Perth/16/09	MDCKX,5	NIVRD	32	64	4	50	43	19	·333
2010	A/HaNam/EL444/10	MDCK3,SIAT1 ^{p}	NxVRD(t|i)	16	64	32	93	**166**	17	·008
2011	A/Victoria/361/11*	MDCK2,SIAT1^{p}	NTVRX(D|G)	32	64	32	25	**67**	20	·000
2012	A/Texas/50/12	C2,MDCK6,SIAT1	NTVHX(D|G)	16	64	32	65	**133**	44	·000
2013	A/Switzerland/9715293/13	SIAT, SIAT8	NTVRD	32	64	64	39	34	21	·132
2014	A/Michigan/15/14	MDCK1, SIAT6	NTVRD	16	40	40	**40**	30	47	·000
2014	A/New Caledonia/104/14	MDCK1, SIAT4^{p}	NTVRX(D|G)	0	64	32	38	36	23	·549
2014	A/HaNam/EL437/14	X,SIAT2	NTVRD	4	32	16	-	-	-	-
2016	A/Newcastle/30/16	SIAT1,SIAT4	NTVRD	8	32	32	47	41	19	·333
2017	A/Kansas/14/17	SIAT3,SIAT1	NTARD	8	32	64	127	101	9	.080
2017	A/Switzerland/8060/17	SIAT2,SIAT2	NTVRD	8	96	9	17	22	5	.373
2018	A/Brisbane/60/18	SIAT3	NTVRD	1	128	128	137	132	18	.331
2004	A/New York/55/04^{egg}	SPFCK3,Egg6	DTVHD	256	256	512	**48**	36	21	·000
2005	A/Wisconsin/67/05^{egg}	SPFCK3, Egg8	DTVHD	64	32	64	**34**	25	20	·001
2007	A/Uruguay/716/07^{egg}	SPFCK1,Egg5	DTVHD	256	128	128	**55**	39	18	·001
2009	A/Perth/16/09^{egg}	Egg6	NTVRD	256	32	32	**56**	24	22	·000
2011	A/Victoria/361/11^{egg}	Egg6	NTVRD	256	256	256	-	-	-	-
2012	A/Texas/50/12^{egg}	Egg5,Egg2	NTVHD	na	128	128	-	-	-	-
2013	A/Switzerland/9715293/13^{egg}	Egg6	NTVRD	512	256	256	37	39	21	·835
2014	A/Hong Kong/4801/14 ^{egg}	Egg7	NTVRD	128	128	128	69	62	45	·260
2017	A/Kansas/14/17 ^{egg}	Egg9	NTARD	-	256	-	-	-	-	-

a: passage cell type followed by number of passages where C = undefined cell line; MDCK = Madin Darby Canine Kidney cell line; SIAT = human 2,6-sialtransferase transfected MDCK cells; P = undefined passage; SPFCK = chicken kidney cell; X = unknown.

b: HA titres were determined against red blood cells from turkeys (T) or guinea pigs (GP)

c: HA and HI titres were determine with and without Osletamivir (OST) at a final concentration of 20 nM

d: Geometric means were calculated and back-transformed. Two-tailed, paired t-test was used to compare titres of each serum sample tested with and without Osletamivir.

* Titrations were also performed with and without oseltamivir on all participant sera (shown below)

p viruses were plaqued on SIAT cells. Plaques that lacked NA 148 and/or 151 substitutions, and consequently lacked NA-mediated agglutination (Table S2, below) were propagated for serology.

Paradoxical effects of OST: no NA 148/151 substitutions, but OST affects HA or HI titre

Partial effect of NA-mediated agglutination: NA 148/151 substitutions and OST reduces HA titre, but has minimal effect on HA titre

Classic NA-mediated agglutination: NA 148/151 substitutions and OST reduces HA titre and increases HI titre

Table S2. Viruses used to generate antisera for antigenic characterization and cartography

Virus	Clade
A/Brisbane/10/2007	
A/Victoria/8/10	1
A/Perth/16/09	1
A/Victoria/8/10	1
A/Victoria/361/11	3c1
A/Texas/50/12	3c1
A/Switz/9715293/13	3c3a
A/HongKong/4801/14e	3c2a
A/Michigan/15/14	3c2a
A/Newcastle/30/16	3c2a1
A/Victoria/653/17	3c2a1b 135K
A/Brisbane/318/16	3c2a2
A/SthAustralia/10/18	3c2a2
A/Brisbane/34/18	3c3a

Selection of erythrocytes for HI assays

HI against A(H3N2) viruses has been performed using Turkey erythrocytes until recently when viruses have agglutinated Turkey erythrocytes poorly necessitating replacement with Guinea Pig erythrocytes (**Table S1**). To avoid experimental variation that may be introduced by using different erythrocytes for past versus recent viruses, we examined whether Guinea Pig erythrocytes can be used instead of Turkey erythrocytes for older viruses. HI titres of 12 anti-sera against 26 viruses isolated between 2004 and 2012 correlated well, and geomteric medians and means were equivalent using Guinea Pig and Turkey erythrocytes (**Figure S3A**). However, HI titres of a panel of 75 human sera (pre-vaccine, post-vaccine and post-season) against A(H3N2) viruses isolated in 2013 and 2014 were substantially lower when using Turkey compared to Guinea Pig erythrocytes (**Figure S3B-E**). Therefore, Guinea Pig erythrocytes were used for all titrations.

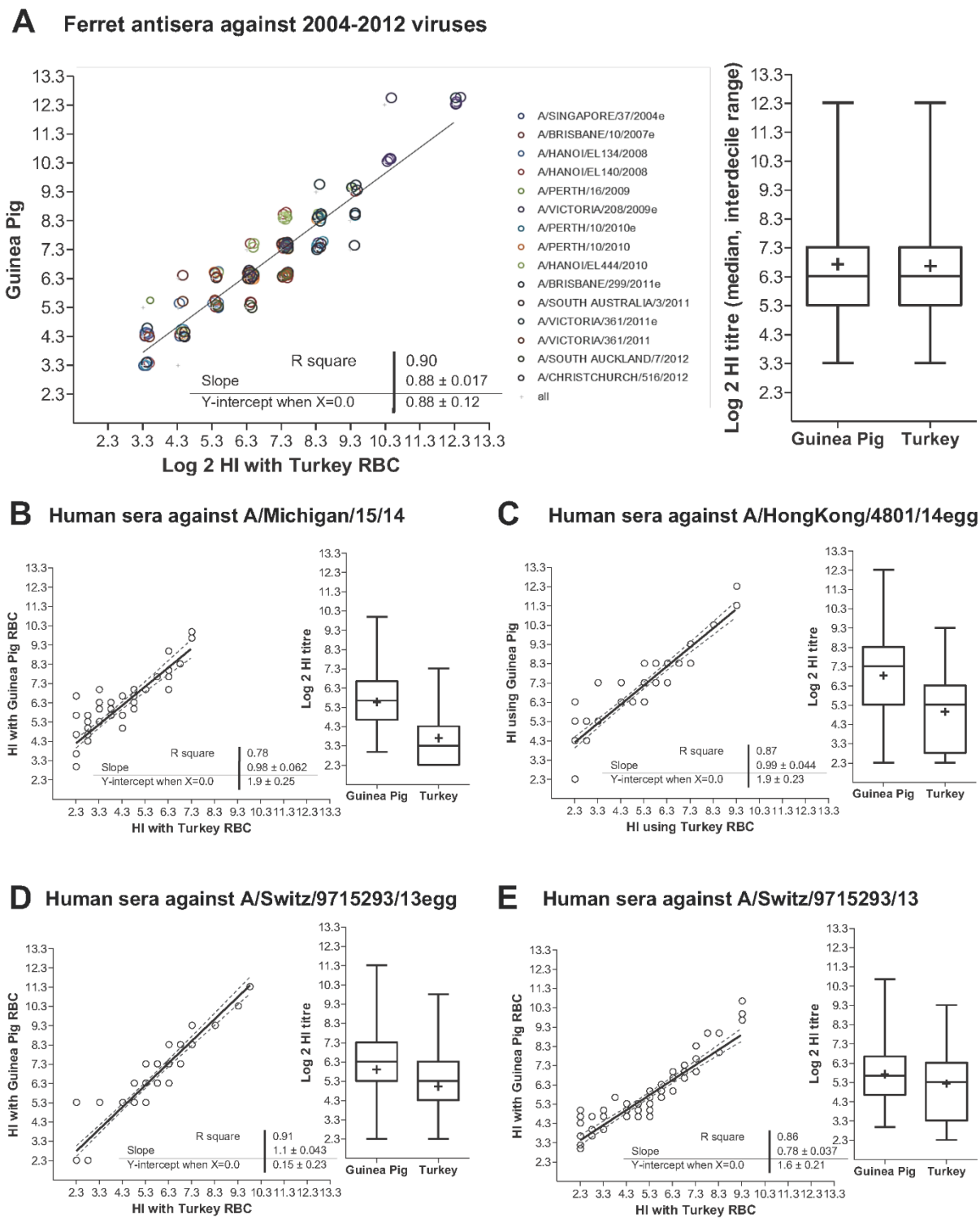

Figure S3. Impact of erythrocyte type on HI titres of ferret first-infection antisera or human sera against A(H3N2) viruses that circulated between 2004 and 2014. Log_2 titres are shown for HI assays performed with Turkey versus Guinea Pig erythrocytes. Left panels show linear regression analysis with 95% confidence intervals. Right panels show summary statistics including median, inter-decile range and range (box and whiskers), and means (+). Ferret anti-sera used in panel A were raised against A/Singapore/37/2004^{egg}, A/Brisbane/10/2007^{egg}, A/Perth/16/2009, A/Victoria/208/2009^{egg}, A/Perth/10/2010, A/Perth/10/2010^{egg}, A/Brisbane/299/2011^{egg}, A/South Australia/3/2011, A/South Auckland/7/2012, A/Christchurch/516/2012, A/Victoria/361/2011^{egg}, and A/Victoria/361/2011. Sera were assessed against 26 viruses of which 15 are shown individually.

Replacement of viruses bearing neuraminidase (NA) mutations that reduce HI assay sensitivity

Some A(H3N2) viruses circulating since 1999 acquired substitutions at positions 148 and/or 151 of NA after passage in MDCK cells (**Table S1**). These substitutions mainly occurred as mixtures such as 148T/I and 151D/N. Substitutions were not detected among viruses passaged only in SIAT cells. HA titres of viruses containing NA 148 and/or 151 substitutions were lower when Oseltamivir, an NA inhibitor, was added indicating that NA was contributing to hemagglutination (**Table S1**), consistent with published studies. [1] HI titrations were performed against a range of older and contemporary strains in the presence and absence of Oseltamivir, and using homologous strain ferret anti-sera as well as human sera (**Table S1**), to determine whether Oseltamivir should be used for all study titrations. Although HI titres against some viruses containing NA 148X or 151X viruses rose when Oseltamivir was added, indicating that NA-mediated agglutination impeded HI antibody detection, there was no effect on titres against other viruses such as A/Brisbane/10/07 and A/Perth/16/09, even though HA titres dropped when Oseltamivir was added. Of concern, titres against a number of viruses were significantly lower in the presence of Oseltamivir. Given that Oseltamivir did not uniformly improve the sensitivity of HI against A(H3N2) viruses, we examined whether NA 148X 151X mixtures could be eliminated by plaquing viruses on SIAT cells (**Table S3**). The majority of plaques selected lacked NA agglutination-associated substitutions, and had relatively high HA and HI titres that were equivalent in the presence and absence of Oseltamivir (**Table S3**, **Figure S4**).

Table S3. NA sequence and titres of viruses after plaque selection on MDCK-SIAT cells

Virus	NA 147-151 sequence	HA PBS	HA OST	HI PBS	HI OST
A/Townsville/2/99	DTVHD	64	64	123	147
A/Philippines/472/02	DTVHD	64	64	123	113
A/Victoria/511/04	DTVHD	48	48	67	73
A/Thailand/409/05	DTVHD	128	128	52	48
A/Brisbane/10/07	DTVRD	128	128	147	160
A/Hanoi/EL134/08	NTVRD	24	24	na	na
A/Perth/16/09	N**T**VRD	64	64	135	147
A/Victoria/361/11*	NTVRD	96	96	*	*
A/New Caledonia/104/14	NTVRD	64	64	87	80

*Titres of all study sera with and without Oseltamivir are summarized in Figure S4 (below).

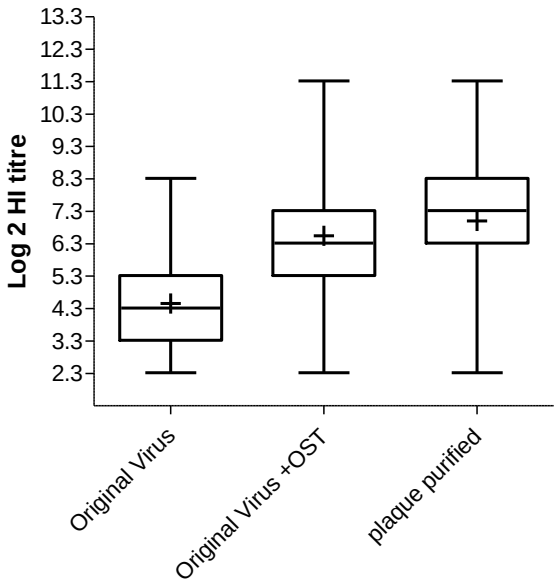

Figure S4. Serum HI titres against A/Victoria/361/2011 are improved by adding Oseltamivir or by plaque selection (on SIAT cells) of virus lacking NA 151G mixtures. Titres are shown for sera from all 100 vaccinees and all time points (baseline and days 4, 7, 14, 21 and 280 post-vaccination). OST: oseltamivir.

Section 3: Numbers of prior A(H3N2) infections detected per participant and year

Table S4. Distribution of prior A(H3N2) virus infections by year and strain

Season	Year	Circulating Strain [a], *equivalent vaccine strain* [b], (**clade**)[c]	Infected once n	Infected twice, n, first (last)	Infected 3 times, n, first (last)	*RTPCR + ILI*
1	2008	A/Ha Nam/EL134/08-like ~ *Br07 + I361R*	2 [d]	5		*2* [e]
2	2009	A/Ha Nam/EL201/09-like ~ *Pe09 + D225N* (**1**)	10 [d]	8 (1) [d]	2	*2* [e]
3	2009	A(H1N1)pdm09 pandemic, A(H3N2) transmission was not detected				
4	2010	A/Ha Nam/EL444/10-like ~ *Pe09 + K144N D53N K62E T212A I230V E280A*	6 [d]	5 (2) [d]		*2* [e]
5	2011	No sequences available	2 [d]	(2) [d]	1	*0*
6	2012	No sequences available	2 [d]	(1) [d]		*0*
7	2012/13	A/Ha Nam/EL12112/12-like ~ *Vi11 + D53N T131K N145S A280E* (**3c1**)	12 [d]	(6) [d]	(1) [d]	*1*
8	2013	A/Ha Nam/EL13132/13-like ~ *Sw13 + N124S S138A S159F D225N R326K* A/Ha Nam/EL13237/13-like ~ *HK14e + S144N Y159F D225N*	4 [d]	(1) [d]		*1*
9	2014	A/Ha Nam/EL14443/14-like ~ *Sw13* (**3c3a**)	9 [d]	(3) [d]		*7* [e]
10	2015	A/Ha Nam/EL15597/15-like ~ *Sw13 + E62K F193S* (**3c3a**) A/Ha Nam/EL15628/15-like ~ *HK14e (***3c2a**)	2 [d]	(2) [d]	(1) [d]	[e]
11	2015	No sequences available	2 [d]		(1) [d]	*1*
Total			**51** [d]	**18** [d]	**3** [d]	

a: Representative of viruses sequenced from swabs of RTPCR-confirmed ILI cases from the Ha Nam Cohort. When more than one clade circulated the most common clade is listed first and the least common is listed last.

b: Vaccine strains with the highest genetic homology to circulating strains are listed followed by any amino acid positions that differed

c: Clade is only indicated for viruses that fall within previously defined clades as indicated on the phylogenetic tree of HA genes shown in figure 2a.

d: Number of participants who were last infected during the indicated season. For columns showing numbers of participants who were infected more than once, the numbers whose last infection was in the indicated season are in shown in parentheses. For example, 11 participants were last infected in 2009 with as A/Ha Nam/EL201/09-like (Pe09) virus, including 10 who were infected once, and 1 who was infected twice and whose last infection was in 2009.

e: One participant had RTPCR-confirmed A(H3N2) virus infection again in season 9 or 10

Section 4: Geometric Mean Titre (GMT) and Geometric Mean Ratio estimates of participants with and without recent A(H3N2) infection

GMTs and GMRs ad 95% confidence intervals were estimated using mixed effects linear regression with a random effects term to account for within-person correlations in the change in antibody titres over time (pre-vaccination, and post-vaccination days 7, 14 and 280). To determine whether estimated GMTs and GMRs varied by prior infection, an interaction term for time of sera collection by prior infection status (prior_H3) was included in the model. Estimates and 95% confidence intervals were back-transformed to titre values for ease of interpretation. The estimated size of the effect of prior A(H3N2) infection on GMT at each time-point was also reported without back-transformation, as such an effect size of 1 represents a two-fold difference in estimated GMTs.

Estimated GMTs against the majority of strains circulating since 2004 were higher among participants who had prior A(H3N2) infection (Table S4), the exception being that prior to vaccination GMTs against post-vaccine strains were similar among participants with and without recent infection. GMTs against strains circulating prior to 2004 were more similar among participants with and without recent A(H3N2) infection (Table S5). The size of the effects of recent A(H3N2) infection on GMTs against strain circulating since 2004 were generally greater post-vaccination than pre-vaccination indicating that recent infection enhanced both pre-vaccine titres and vaccine-induced titre boosting. GMRs were higher on day 14 post-vaccine than at earlier and later time-points (Table S6), and were highest against HK14e and strains proximal to HK14e. GMRs against the majority of viruses were substantially but not significantly higher among participants with recent infection.

Table S5 Geometric Mean Titres against 2004-2018 A(H3N2) virus strains

	GMT (95% CI)								Effect Size of Prior H3N2			
	Pre-Vaccine		Day 7		Day 14		Day 280					
Virus	No Prior	Prior H3N2	No Prior	Prior H3N2	No Prior	Prior H3N2	No Prior	Prior H3N2	Pre-vaccine	d7	d14	d280
NY04e	26 (18, 37)	43 (34, 54)	64 (44, 93)	110 (87, 139)	102 (70, 149)	163 (129, 206)	46 (32, 67)	86 (68, 108)	0.7 (0.1, 1.4)	0.7 (0.1, 1.4)	0.7 (0.0, 1.3)	0.9 (0.3, 1.5)
Vi04	38 (29, 50)	58 (49, 68)	84 (64, 110)	129 (109, 153)	100 (76, 131)	158 (133, 188)	65 (49, 85)	100 (84, 119)	0.6 (0.1, 1.1)	0.6 (0.2, 1.1)	0.7 (0.2, 1.1)	0.6 (0.2, 1.2)
Wi05e	24 (16, 35)	42 (33, 53)	58 (39, 86)	116 (91, 149)	102 (69, 152)	183 (143, 234)	50 (33, 74)	91 (71, 117)	0.8 (0.1, 1.5)	1.0 (0.3, 1.7)	0.8 (0.2, 1.5)	0.9 (0.2, 1.6)
Th05	28 (21, 38)	42 (35, 50)	57 (42, 76)	101 (84, 121)	80 (60, 107)	124 (104, 149)	50 (37, 67)	76 (64, 92)	0.6 (0.1, 1.1)	0.8 (0.3, 1.3)	0.6 (0.1, 1.1)	0.6 (0.1, 1.1)
Ur07e	21 (14, 31)	36 (27, 46)	66 (43, 100)	127 (98, 165)	119 (78, 180)	201 (155, 261)	47 (31, 71)	93 (71, 120)	0.8 (0.1, 1.5)	1.0 (0.2, 1.7)	0.8 (0.1, 1.5)	1.0 (0.3, 1.7)
Br07	37 (28, 49)	65 (55, 77)	57 (43, 74)	138 (117, 164)	82 (62, 108)	173 (145, 205)	58 (44, 76)	118 (99, 140)	0.8 (0.3, 1.3)	1.3 (0.8, 1.8)	1.1 (0.6, 1.5)	1.0 (0.6, 1.5)
HN08	30 (22, 39)	44 (37, 53)	51 (39, 68)	96 (80, 115)	62 (47, 83)	115 (97, 138)	43 (32, 57)	76 (64, 91)	0.6 (0.1, 1.1)	0.9 (0.4, 1.4)	0.9 (0.4, 1.4)	0.8 (0.3, 1.3)
Pe09	31 (23, 43)	60 (50, 73)	78 (57, 106)	149 (123, 181)	102 (75, 140)	207 (171, 251)	55 (40, 75)	128 (106, 156)	1.0 (0.4, 1.5)	0.9 (0.4, 1.5)	1.0 (0.5, 1.5)	1.2 (0.7, 1.8)
Pe09e	17 (12, 25)	32 (25, 40)	55 (38, 81)	127 (100, 161)	105 (71, 154)	215 (169, 274)	31 (21, 45)	90 (71, 115)	0.9 (0.2, 1.5)	1.2 (0.6, 1.9)	1.0 (0.4, 1.7)	1.6 (0.9, 2.2)
HN09	30 (23, 41)	54 (45, 65)	80 (60, 107)	160 (133, 192)	134 (100, 180)	230 (192, 277)	63 (47, 84)	125 (104, 150)	0.8 (0.3, 1.3)	1.0 (0.5, 1.5)	0.8 (0.3, 1.3)	1.0 (0.5, 1.5)
HN10	30 (22, 41)	61 (50, 75)	82 (59, 113)	181 (148, 222)	134 (97, 186)	244 (199, 299)	63 (45, 87)	137 (112, 168)	1.0 (0.5, 1.6)	1.1 (0.6, 1.7)	0.9 (0.3, 1.4)	1.1 (0.6, 1.7)
Vi11e	50 (34, 73)	94 (74, 119)	152 (104, 223)	342 (269, 434)	297 (202, 435)	645 (509, 819)	103 (70, 151)	241 (190, 307)	0.9 (0.3, 1.6)	1.2 (0.5, 1.8)	1.1 (0.5, 1.8)	1.2 (0.6, 1.9)
Vi11	29 (22, 38)	60 (51, 71)	74 (57, 96)	155 (132, 183)	125 (96, 162)	194 (165, 228)	70 (54, 91)	146 (124, 172)	1.1 (0.6, 1.5)	1.1 (0.6, 1.5)	0.6 (0.2, 1.1)	1.1 (0.6, 1.5)
Tx12	32 (22, 47)	92 (72, 118)	138 (93, 204)	418 (328, 534)	289 (196, 428)	664 (520, 848)	111 (75, 164)	318 (249, 407)	1.5 (0.9, 2.2)	1.6 (0.9, 2.3)	1.2 (0.5, 1.9)	1.5 (0.9, 2.2)
Tx12e	42 (28, 63)	79 (62, 102)	145 (97, 217)	335 (261, 431)	282 (189, 422)	603 (469, 776)	105 (70, 157)	239 (186, 308)	0.9 (0.2, 1.6)	1.2 (0.5, 1.9)	1.1 (0.4, 1.8)	1.2 (0.5, 1.9)
Sw13e	20 (13, 29)	34 (27, 44)	59 (40, 88)	122 (96, 155)	93 (63, 137)	192 (151, 244)	32 (22, 47)	83 (65, 106)	0.8 (0.2, 1.5)	1.0 (0.4, 1.7)	1.1 (0.4, 1.7)	1.4 (0.7, 2.0)
Sw13	15 (10, 23)	28 (22, 36)	45 (30, 68)	122 (95, 157)	82 (54, 123)	209 (162, 270)	30 (20, 46)	85 (66, 109)	0.9 (0.2, 1.6)	1.4 (0.7, 2.1)	1.4 (0.7, 2.0)	1.5 (0.8, 2.2)
HN14	12 (8, 17)	23 (18, 29)	41 (28, 60)	114 (90, 144)	76 (52, 111)	209 (166, 264)	35 (24, 51)	80 (63, 101)	0.9 (0.3, 1.6)	1.4 (0.8, 2.1)	1.5 (0.8, 2.1)	1.2 (0.6, 1.8)
HK14e	23 (15, 34)	42 (32, 53)	97 (65, 146)	217 (169, 280)	210 (140, 314)	407 (316, 523)	57 (38, 86)	164 (127, 211)	0.9 (0.2, 1.6)	1.2 (0.5, 1.9)	1.0 (0.3, 1.6)	1.5 (0.8, 2.2)
Mi14	11 (8, 16)	20 (16, 25)	37 (26, 54)	95 (76, 119)	69 (48, 99)	181 (144, 228)	32 (22, 46)	67 (53, 84)	0.9 (0.3, 1.5)	1.4 (0.7, 2.0)	1.4 (0.8, 2.0)	1.1 (0.5, 1.7)
NC14	15 (11, 20)	30 (25, 36)	39 (29, 52)	87 (73, 104)	66 (49, 88)	122 (102, 146)	30 (22, 40)	69 (58, 83)	1.0 (0.5, 1.5)	1.2 (0.7, 1.7)	0.9 (0.4, 1.4)	1.2 (0.7, 1.7)
NC16	13 (9, 19)	25 (20, 31)	45 (32, 65)	98 (78, 122)	78 (54, 112)	174 (139, 218)	28 (20, 41)	67 (54, 84)	0.9 (0.3, 1.5)	1.1 (0.5, 1.7)	1.2 (0.6, 1.8)	1.3 (0.6, 1.9)
Sw17	7 (6, 9)	9 (8, 10)	13 (10, 16)	19 (16, 21)	15 (12, 19)	22 (20, 26)	10 (8, 12)	15 (14, 18)	0.3 (-0.1, 0.7)	0.6 (0.2, 0.9)	0.6 (0.2, 0.9)	0.6 (0.3, 1.0)
Ka17	9 (7, 13)	14 (11, 17)	21 (15, 30)	45 (36, 56)	33 (23, 47)	77 (61, 97)	11 (8, 16)	28 (22, 36)	0.5 (-0.1, 1.2)	1.1 (0.5, 1.7)	1.2 (0.6, 1.9)	1.4 (0.7, 2.0)
Ka17e	7 (5, 11)	9 (7, 11)	22 (15, 32)	39 (31, 50)	31 (21, 46)	65 (51, 83)	8 (6, 12)	22 (17, 28)	0.3 (-0.4, 0.9)	0.9 (0.2, 1.5)	1.1 (0.4, 1.7)	1.4 (0.8, 2.1)
Br18	20 (15, 26)	28 (23, 33)	43 (32, 58)	86 (71, 103)	64 (48, 86)	128 (106, 154)	35 (26, 47)	63 (52, 76)	0.5 (-0.0, 1.0)	1.0 (0.5, 1.5)	1.0 (0.5, 1.5)	0.9 (0.3, 1.4)

GMT estimates are shaded when confidence intervals for participants with prior A(H3N2) infection did not overlap with those of participants with no recent prior A(H3N2) infection.
Effect size estimates are shaded when confidence intervals did not cross 0.
Boxes indicate viruses against which titres were highest at that time-point, considering pre-2004 strains shown in **Table S5**

Table S6. Geometric Mean Titres against 1968-2002 A(H3N2) virus strains

	GMT (95% CI)						Effect size (95% CI)	
	Pre-vaccine		Day 14		Day 280			
Virus	No Prior H3	Prior H3	No Prior H3	Prior H3	No Prior H3	Prior H3	Day 14	Day 280
Bi68	40.0 (29.6, 53.9)	47.1 (39.01, 56.74)	49.9 (37.0, 67.4)	64.0 (53.1, 77.2)	48.8 (36.1, 66.0)	54.3 (45.0, 65.5)	0.36 (-0.15, 0.87)	0.15 (-0.36, 0.67)
Bi72	49.9 (35.3, 70.6)	68.5 (55.2, 85.0)	63.9 (45.2, 90.4)	94.1 (75.8, 116.8)	55.0 (38.9, 77.9)	80.8 (65.0, 100.3)	0.56 (-0.03, 1.15)	0.55 (-0.04, 1.14)
Bi76	32.8 (22.7, 47.3)	34.6 (27.5, 43.5)	44.1 (30.6, 63.6)	49.4 (39.3, 62.0)	33.6 (23.3, 48.5)	41.0 (32.6, 51.5)	0.16 (-0.46, 0.79)	0.29 (-0.34, 0.91)
Ne82	25.6 (18.6, 35.2)	29.9 (24.5, 36.5)	34.4 (25.1, 47.3)	45.7 (37.5, 55.7)	28.9 (21.0, 39.8)	36.9 (30.2, 45.0)	0.41 (-0.13, 0.95)	0.35 (-0.19, 0.89)
Ph82	39.0 (27.7, 54.9)	51.8 (41.8, 64.2)	55.1 (39.1, 77.7)	79.1 (63.9, 98.0)	52.1 (37.0, 73.6)	67.0 (54.0, 83.0)	0.52 (-0.06, 1.11)	0.36 (-0.22, 0.95)
Ne89	46.3 (33.8, 63.6)	59.9 (49.1, 72.9)	76.3 (55.4, 104.4)	99.7 (81.8, 121.5)	69.1 (50.3, 95.0)	84.3 (69.1, 102.8)	0.39 (-0.15, 0.93)	0.29 (-0.25, 0.83)
Ne92	28.3 (20.9, 38.2)	36.3 (30.1, 43.8)	45.2 (33.5, 61.1)	71.2 (59.0, 85.9)	38.7 (28.6, 52.4)	53.0 (43.9, 64.0)	0.65 (0.14, 1.17)	0.45 (-0.06, 0.97)
Ne93	68.9 (47.6, 99.6)	92.3 (73.3, 116.2)	121.7 (84.1, 176.0)	179.4 (142.5, 225.8)	79.4 (54.8, 115.0)	141.4 (112.3, 178.2)	0.56 (-0.07, 1.19)	0.83 (0.2, 1.46)
Ne95	56.5 (39.3, 81.3)	95.0 (75.7, 119.2)	141.2 (98.1, 203.1)	186.4 (148.6, 233.9)	87.5 (60.7, 126.0)	146.2 (116.4, 183.5)	0.4 (-0.22, 1.02)	0.74 (0.12, 1.36)
Ta97	35.3 (25.5, 49.0)	46.2 (37.6, 56.6)	86.1 (62.0, 119.4)	98.7 (80.5, 121.1)	51.5 (37.1, 71.6)	68.4 (55.7, 83.9)	0.2 (-0.36, 0.75)	0.41 (-0.15, 0.97)
TV99	55.1 (44.1, 68.9)	76.7 (66.7, 88.2)	113.0 (90.4, 141.2)	137.0 (119.2, 157.4)	80.4 (64.3, 100.7)	117.3 (102.0, 134.9)	0.28 (-0.1, 0.66)	0.54 (0.16, 0.93)
Ne99	68.9 (47.5, 99.9)	89.68 (71.1, 113.1)	190.0 (131.0, 275.7)	209.2 (165.9, 263.9)	113.8 (78.3, 165.4)	131.4 (104.1, 165.9)	0.14 (-0.49, 0.77)	0.21 (-0.43, 0.84)
Ph02	74.2 (57.7, 95.4)	88.8 (75.9, 103.9)	159.8 (124.2, 205.5)	201.3 (172.1, 235.5)	115.7 (89.8, 149.1)	135.4 (115.6, 158.6)	0.33 (-0.09, 0.76)	0.23 (-0.2, 0.66)
Fu02	88.2 (65.6, 118.6)	134.4 (111.7, 161.6)	255.8 (190.2, 343.8)	316.5 (263.2, 380.7)	146.4 (108.7, 197.2)	204.3 (169.7, 246.0)	0.31 (-0.2, 0.81)	0.48 (-0.03, 0.99)

GMT estimates are shaded when confidence intervals for participants with prior A(H3N2) infection did not overlap with those of participants with no recent prior A(H3N2) infection.
Effect size estimates are shaded when confidence intervals did not cross 0.
Boxes indicate viruses against which titres were highest at that time-point, considering post-2003 strains shown in **Table S4**

Table S7. Geometric Mean Ratios against A(H3N2) viruses circulating between 1968 and 2018

Virus	Day 7		Day 14		Day 280	
	No Prior H3	Prior H3	No Prior H3	Prior H3	No Prior H3	Prior H3
Bi68	1.1 (0.9, 1.3)	1.2 (1.1, 1.4)	1.3 (1.1, 1.5)	1.4 (1.2, 1.5)	1.2 (1.0, 1.4)	1.2 (1.1, 1.3)
Bi72	1.2 (1.0, 1.4)	1.3 (1.1, 1.4)	1.3 (1.1, 1.5)	1.4 (1.2, 1.5)	1.1 (0.9, 1.3)	1.2 (1.1, 1.3)
Bi76	1.3 (1.1, 1.6)	1.5 (1.4, 1.7)	1.2 (1.0, 1.4)	1.3 (1.2, 1.5)	1.2 (1.0, 1.4)	1.3 (1.2, 1.5)
Ne82	1.2 (1.0, 1.4)	1.5 (1.3, 1.6)	1.4 (1.1, 1.6)	1.5 (1.4, 1.7)	1.1 (1.0, 1.3)	1.2 (1.1, 1.4)
Ph82	1.4 (1.1, 1.6)	1.5 (1.3, 1.6)	1.4 (1.2, 1.7)	1.5 (1.4, 1.7)	1.3 (1.1, 1.6)	1.3 (1.2, 1.4)
Ne89	1.4 (1.1, 1.7)	1.5 (1.3, 1.7)	1.6 (1.3, 2.0)	1.7 (1.5, 1.9)	1.5 (1.2, 1.8)	1.4 (1.2, 1.6)
Ne92	1.5 (1.2, 1.8)	1.7 (1.5, 2.0)	1.6 (1.3, 2.0)	2.0 (1.7, 2.3)	1.4 (1.1, 1.7)	1.5 (1.3, 1.7)
Ne93	1.5 (1.2, 1.9)	1.8 (1.5, 2.1)	1.8 (1.4, 2.2)	1.9 (1.7, 2.3)	1.2 (0.9, 1.5)	1.5 (1.3, 1.8)
Ne95	1.7 (1.3, 2.1)	1.7 (1.5, 2.0)	2.5 (2.0, 3.2)	2.0 (1.7, 2.3)	1.6 (1.2, 2.0)	1.5 (1.3, 1.8)
Ta97	1.7 (1.4, 2.2)	1.9 (1.6, 2.1)	2.4 (1.9, 3.1)	2.1 (1.9, 2.5)	1.5 (1.2, 1.8)	1.5 (1.3, 1.7)
TV99	1.6 (1.3, 1.9)	1.6 (1.5, 1.8)	2.1 (1.7, 2.5)	1.8 (1.6, 2.0)	1.5 (1.2, 1.7)	1.5 (1.4, 1.7)
Ne99	1.6 (1.2, 2.0)	1.8 (1.6, 2.2)	2.8 (2.1, 3.6)	2.3 (2.0, 2.7)	1.6 (1.3, 2.1)	1.5 (1.3, 1.7)
Ph02	1.6 (1.3, 2.0)	1.8 (1.6, 2.1)	2.2 (1.8, 2.7)	2.3 (2.0, 2.6)	1.6 (1.3, 1.9)	1.5 (1.3, 1.7)
Fu02	2.1 (1.6, 2.6)	1.8 (1.5, 2.1)	2.9 (2.3, 3.7)	2.4 (2.0, 2.7)	1.7 (1.3, 2.1)	1.5 (1.3, 1.8)
NY04e	2.5 (1.9, 3.3)	2.6 (2.2, 3.1)	4.0 (3.0, 5.3)	3.8 (3.2, 4.6)	1.8 (1.4, 2.4)	2.0 (1.7, 2.4)
Vi04	2.2 (1.8, 2.7)	2.2 (2.0, 2.6)	2.6 (2.1, 3.3)	2.8 (2.4, 3.1)	1.7 (1.4, 2.1)	1.7 (1.5, 2.0)
Wi05e	2.4 (1.8, 3.3)	2.8 (2.3, 3.4)	4.3 (3.2, 5.9)	4.4 (3.6, 5.3)	2.1 (1.5, 2.9)	2.2 (1.8, 2.7)
Th05	2.0 (1.6, 2.5)	2.4 (2.1, 2.8)	2.8 (2.3, 3.5)	3.0 (2.6, 3.4)	1.8 (1.4, 2.2)	1.8 (1.6, 2.1)
Ur07e	3.2 (2.2, 4.6)	3.6 (2.8, 4.5)	5.8 (4.0, 8.3)	5.7 (4.5, 7.1)	2.3 (1.6, 3.3)	2.6 (2.1, 3.3)
Br07	1.5 (1.2, 1.9)	2.1 (1.9, 2.4)	2.2 (1.8, 2.7)	2.7 (2.4, 3.0)	1.6 (1.3, 1.9)	1.8 (1.6, 2.1)
HN08	1.7 (1.4, 2.2)	2.2 (1.9, 2.5)	2.1 (1.7, 2.6)	2.6 (2.3, 3.0)	1.4 (1.2, 1.8)	1.7 (1.5, 2.0)
Pe09	2.5 (1.9, 3.3)	2.5 (2.1, 2.9)	3.3 (2.5, 4.3)	3.4 (2.9, 4.0)	1.8 (1.3, 2.3)	2.1 (1.8, 2.5)
Pe09e	3.2 (2.1, 4.8)	4.0 (3.1, 5.2)	6.1 (4.0, 9.2)	6.8 (5.3, 8.8)	1.8 (1.2, 2.7)	2.9 (2.2, 3.7)
HN09	2.6 (2.0, 3.5)	3.0 (2.5, 3.5)	4.4 (3.4, 5.8)	4.3 (3.6, 5.1)	2.1 (1.6, 2.7)	2.3 (1.9, 2.7)
HN10	2.8 (2.1, 3.7)	3.0 (2.5, 3.6)	4.5 (3.4, 6.1)	4.0 (3.3, 4.8)	2.1 (1.6, 2.8)	2.3 (1.9, 2.7)
Vi11e	3.1 (2.1, 4.5)	3.7 (2.9, 4.7)	5.9 (4.0, 8.8)	6.9 (5.4, 8.9)	2.0 (1.4, 3.0)	2.6 (2.0, 3.3)
Vi11	2.6 (2.0, 3.3)	2.6 (2.2, 3.0)	4.3 (3.4, 5.5)	3.2 (2.7, 3.7)	2.4 (1.9, 3.1)	2.4 (2.1, 2.8)
Tx12	4.3 (2.9, 6.3)	4.5 (3.6, 5.7)	9.1 (6.2, 13.2)	7.2 (5.7, 9.1)	3.4 (2.4, 5.1)	3.5 (2.7, 4.4)

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Name	Day 7		Day 14		Day 280	
	No Prior H3	Prior H3	No Prior H3	Prior H3	No Prior H3	Prior H3
Tx12e	3.5 (2.2, 5.4)	4.2 (3.2, 5.6)	6.7 (4.3, 10.5)	7.6 (5.8, 10.1)	2.5 (1.6, 3.9)	3.1 (2.3, 4.0)
Sw13e	3.1 (2.0, 4.5)	3.6 (2.8, 4.6)	4.8 (3.2, 7.1)	5.6 (4.4, 7.2)	1.6 (1.1, 2.4)	2.4 (1.9, 3.1)
Sw13	3.0 (2.0, 4.5)	4.3 (3.4, 5.6)	5.4 (3.6, 8.1)	7.4 (5.8, 9.5)	2.0 (1.3, 3.0)	3.0 (2.3, 3.9)
HN14	3.5 (2.4, 5.0)	5.0 (4.0, 6.4)	6.4 (4.4, 9.3)	9.2 (7.3, 11.7)	2.9 (2.0, 4.2)	3.6 (2.8, 4.5)
HK14e	4.3 (2.7, 6.9)	5.2 (3.9, 7.0)	9.3 (5.8, 14.8)	9.8 (7.3, 13.1)	2.5 (1.6, 4.0)	4.0 (3.0, 5.3)
Mi14	3.5 (2.3, 5.1)	4.8 (3.7, 6.1)	6.4 (4.3, 9.5)	9.1 (7.1, 11.6)	2.9 (2.0, 4.3)	3.4 (2.6, 4.3)
NC14	2.6 (2.0, 3.3)	2.9 (2.5, 3.5)	4.3 (3.3, 5.6)	4.1 (3.5, 4.9)	2.0 (1.5, 2.6)	2.4 (2.0, 2.8)
NC16	3.4 (2.3, 4.9)	3.9 (3.1, 5.0)	5.8 (4.0, 8.5)	7.0 (5.5, 8.9)	2.1 (1.4, 3.1)	2.7 (2.2, 3.5)
Sw17	1.8 (1.4, 2.2)	2.1 (1.9, 2.4)	2.1 (1.7, 2.6)	2.5 (2.2, 2.9)	1.4 (1.1, 1.7)	1.7 (1.5, 2.0)
Ka17	2.3 (1.6, 3.3)	3.3 (2.6, 4.2)	3.5 (2.4, 5.1)	5.7 (4.5, 7.2)	1.2 (0.8, 1.7)	2.1 (1.6, 2.6)
Ka17e	2.9 (1.9, 4.5)	4.5 (3.4, 5.8)	4.2 (2.7, 6.5)	7.4 (5.6, 9.7)	1.1 (0.7, 1.7)	2.5 (1.9, 3.3)
Br18	2.2 (1.6, 3.0)	3.1 (2.6, 3.8)	3.3 (2.4, 4.5)	4.7 (3.9, 5.7)	1.8 (1.3, 2.4)	2.3 (1.9, 2.8)

Section 5: Titres and titre increments by age

Participants with and without recent A(H3N2) infection were of similar ages. Nevertheless, to determine if age may affect the titre and strain coverage of antibodies induced by vaccination, we used Generalized Additive Models (GAMs) to visualize the distribution of titres and titre increments as contours by virus circulation year versus participant age (**Figure S7**). Pre-vaccine titres were highest against viruses circulating between approximately 1990 to 2000. Participants who had recent infection had higher pre-vaccine titres than those who lacked recent infection, particularly against strains circulating between 2000 and 2010, and most prominently among participants aged below 35 years, followed by those aged over 65 years. Otherwise the distribution of titres across strains by participant age was similar for those with and without recent infection. Vaccine-induced titre increment by day 7 was greatest against strains circulating after 2010 and was slightly greater among participants who had recent infection, but with a similar distribution of titre increment by age compared to participants without recent infection indicating that effects of recent infection are not age dependent. Participants aged below 25 had negligible titre increment against older strains, as may be expected for strains that circulated prior to their birth. Trends for titre increment on day 14 were similar to day 7 with the exception that increments were higher overall and were now clearly higher among the older compared to younger participants, both with and without recent infection. Similarly, titres at day 14 across strains were highest amongst the oldest participants, whether or not they had recent infection. Trends for titre increment by day 21 were similar to day 14, with the main exception that increments were lower overall, and were now more clearly higher among participants with recent infection, a trend that persisted at day 280. Consequently, at day 280 post-vaccination titres across strains differed more between participants with and without recent infection than observed pre-vaccine. In particular, participants of all ages who had recent infection had higher titres against strains circulating between ~2005 and 2015, than participants who lacked recent infection. As with pre-vaccine titres, day 280 titres were highest in the youngest and oldest ages, regardless of recent infection. In summary, the results indicate that vaccine-induced antibody production was enhanced by recent infection and by older age.

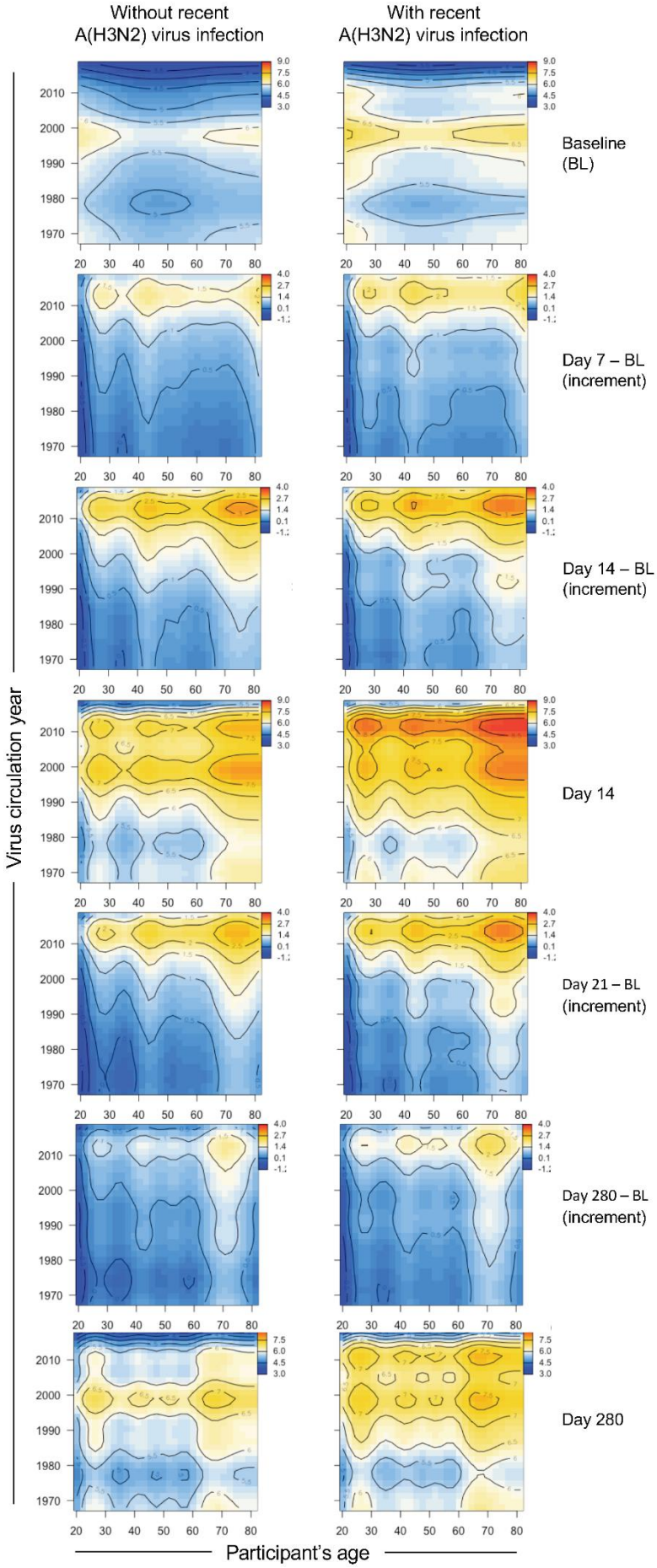


Figure S7. Antibody titres across age groups in participants with and without A(H3N2) virus infection since 2007. Contours depict titres and titre increments by strain and participant age fitted using GAMs. Participants with (n=72) and without (n=28) recent A(H3N2) virus infection are compared. 40 A(H3N2) strains are represented by year of first detection, ranging from 1968 to 2018. Panels are arranged horizontally by vaccine time point, and either show titres at that time point or titre increment compared to baseline.

Section 6: **Incremental changes in landscapes between post-vaccination time points**

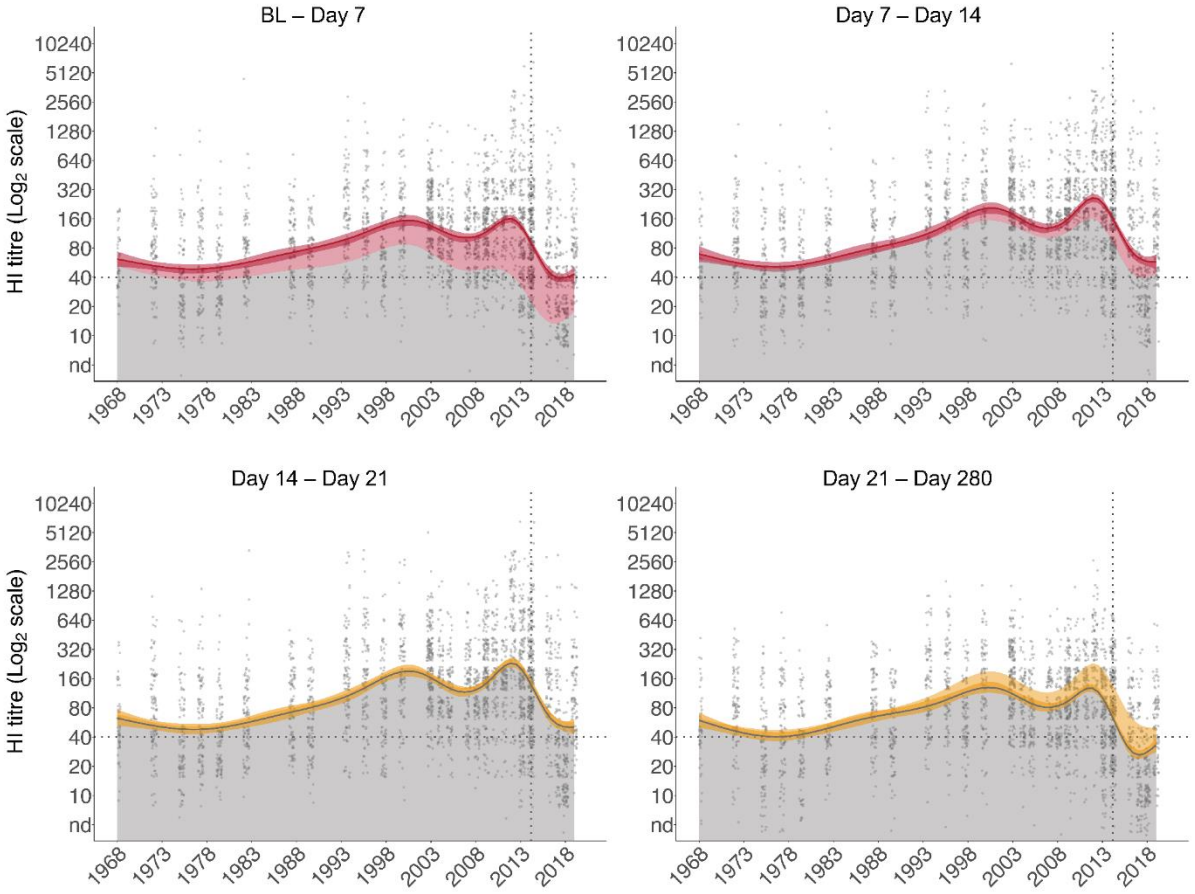


Figure S8. Incremental change in antibody landscapes at each study time point. Lines represent titre landscapes across strains estimated using GAMs. Titre landscapes at the first time point in each panel are shown in grey and landscapes at the subsequent time point are shown in red if higher and in yellow if lower than at the earlier time point. Dark shading either side of the lines indicates the 95% CIs for the model, and dots show individual participant titres against each antigen. Light shaded areas reflect the difference in the landscapes between the time points. BL: baseline.

Section 7: **Titres landscape 280 days after vaccination compared to baseline**

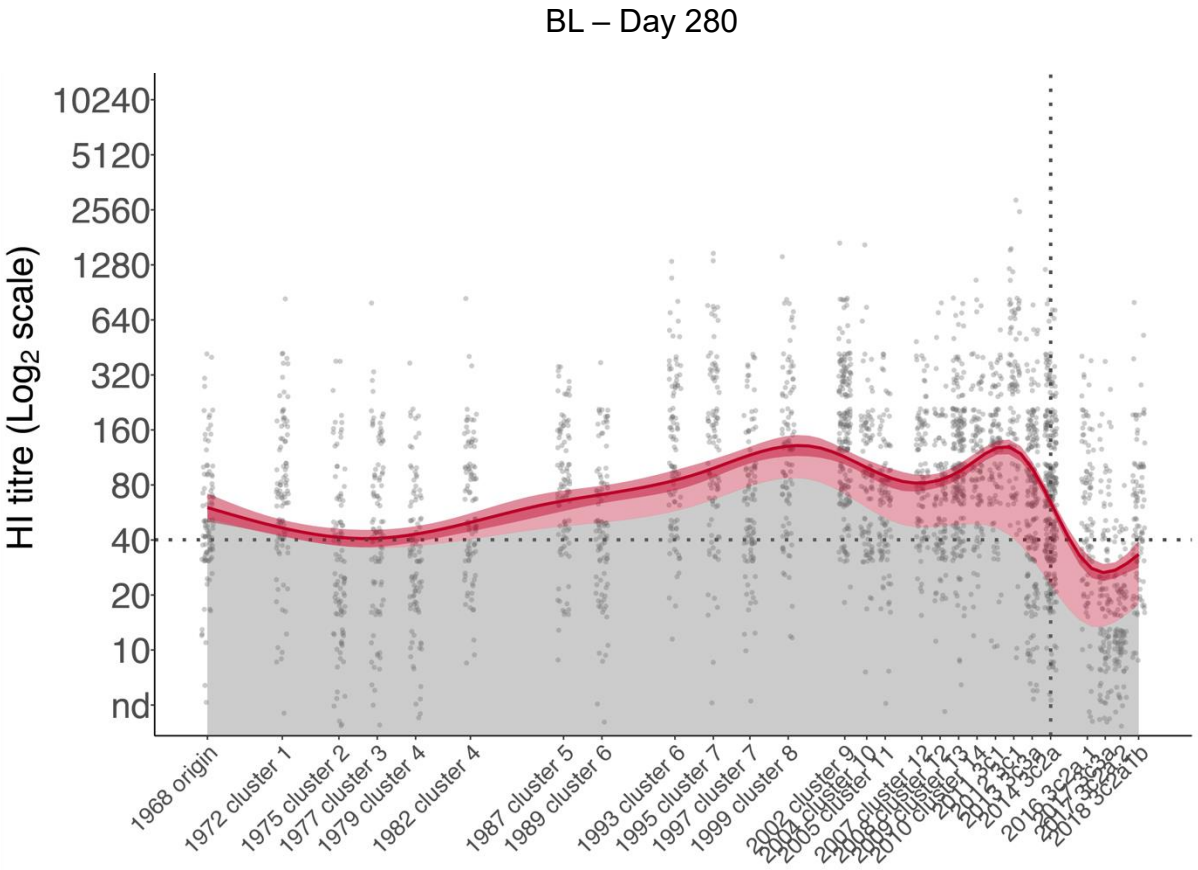


Figure S9. Antibody titres against a large range of A(H3N2) strains are higher 280 days after vaccination than in pre-vaccination sera. Antibody landscapes prior to vaccination (BL) are shown in grey and landscapes 280 days after vaccination are shown in red. Antibody titres across multiple strains were estimated, and are presented, as in Figure S8.

Section 8: **Effects of prior infecting clade on antibodies induced by HK14e vaccine**

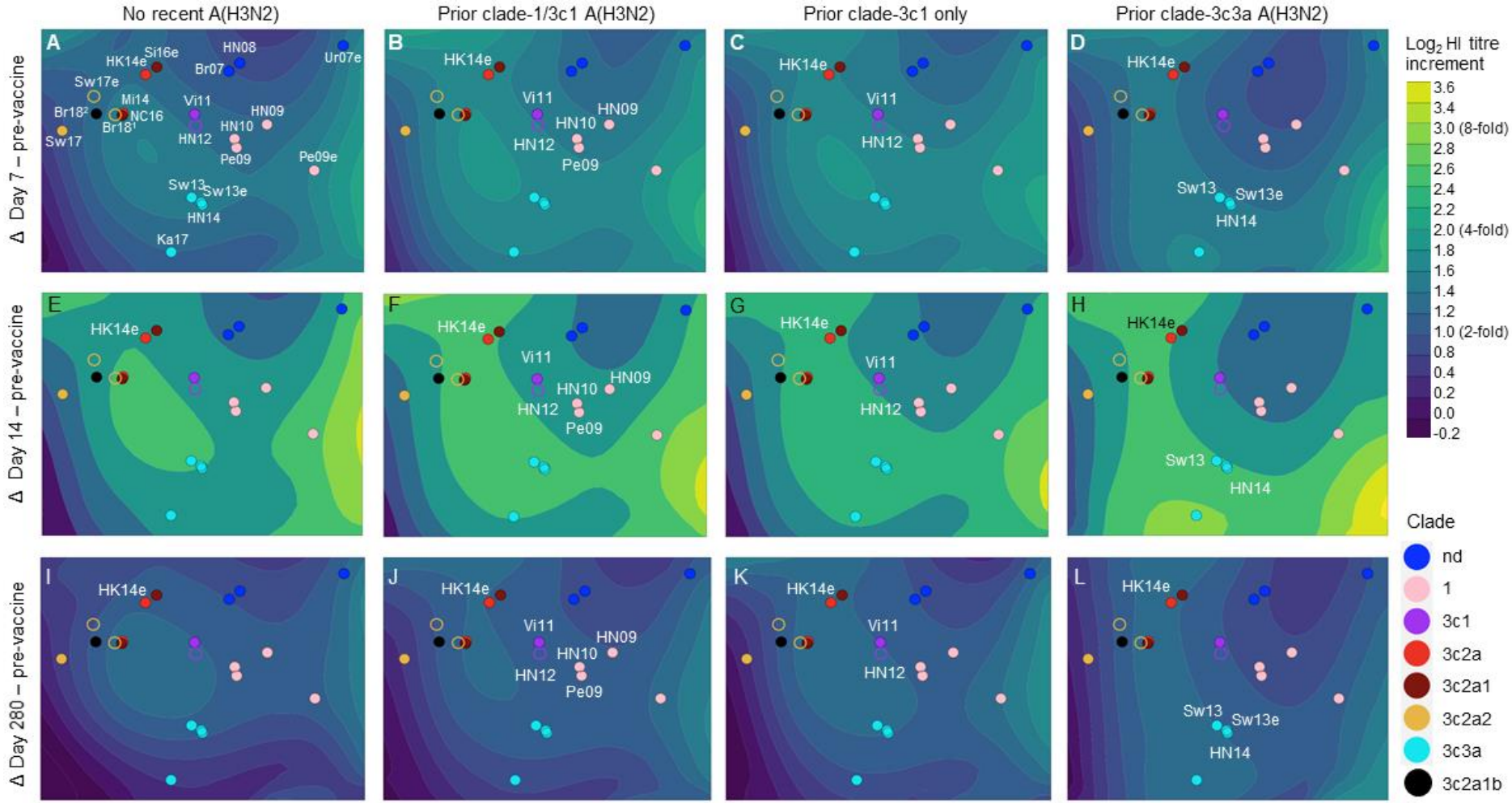
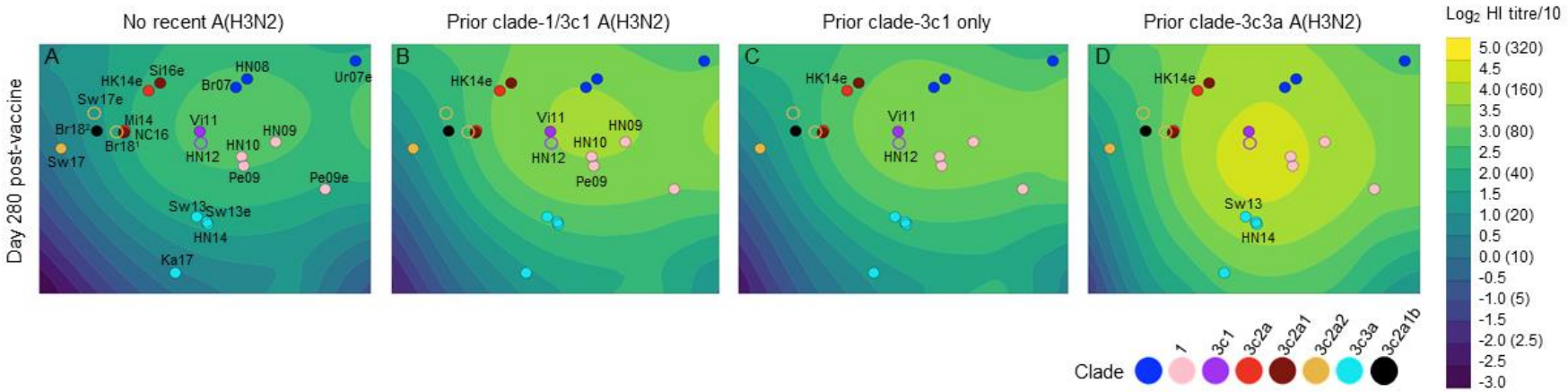


Figure S10. Effects of prior A(H3N2) infection and infecting clade on the strain coverage of antibodies detected 7 and 280 days after vaccination. Landscape plots, constructed as in Figure 5, of titres and titre increments show that effects of prior infection, and of the clade causing infection, on the strain-coverage of antibodies induced by vaccination can be detected by day 7 and are maintained until day 280 after vaccination.


No recent A(H3N2)
Prior clade-1/3c1 A(H3N2)
Prior clade-3c1 only
Prior clade-3c3a A(H3N2)
Day 280 post-vaccine
A
B
C
D
HK14e
Si16e
HN08
Br07
Ur07e
Sw17e
Mi14
NC16
Vi11
HN09
Br18²
Br18¹
HN10
HN12
Sw17
Pe09
Pe09e
Sw13
Sw13e
HN14
Ka17
Clade
1
3c1
3c2a
3c2a1
3c2a2
3c3a
3c2a1b
Log₂ HI titre/10
5.0 (320)
4.5
4.0 (160)
3.5
3.0 (80)
2.5
2.0 (40)
1.5
1.0 (20)
0.5
0.0 (10)
-0.5
-1.0 (5)
-1.5
-2.0 (2.5)
-2.5
-3.0

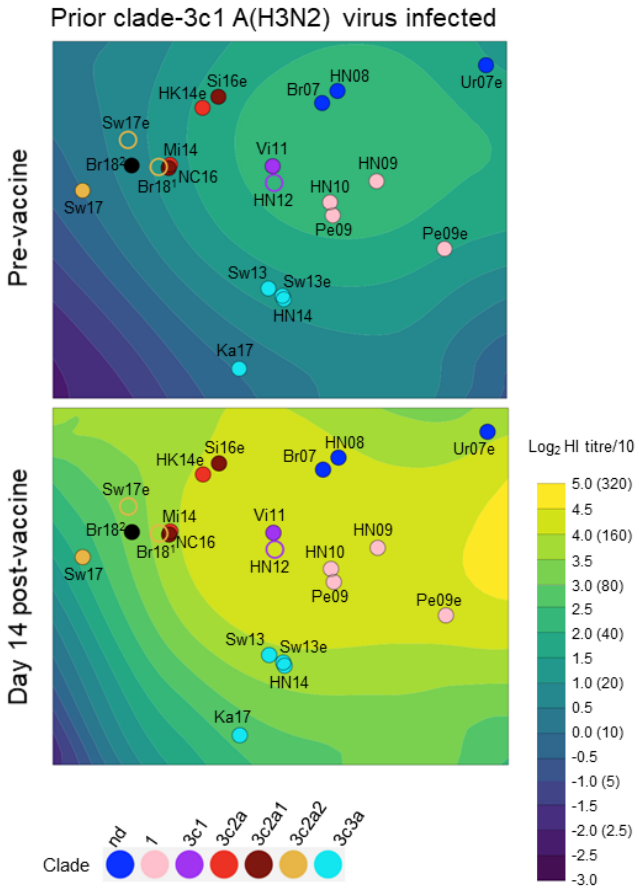


Figure S11. Strain coverage of antibodies in pre- and post-vaccination sera of participants who had prior infection with a clade-3c1 virus (HN12/Vi11-like). Landscape plots, constructed as in Figure 5, are shown separately for 19 participants who were last infected in 2012 (season 7) when clade-3c1 viruses, such as HN12, circulated. Landscapes of pre- and day 14 post-vaccination titres are similar to those of clade-1 and clade-3c1 infected participants combined (Figure 5).

Generation and validation of antigenic site B mutant HK14e virus

Reverse genetics (RG) viruses were produced using PHW2000 plasmids encoding internal genes and NA of A/Puerto Rico/8/1934 (PR8) and HA of egg-grown A/Hong Kong/4801/2014 (HK14e) following protocols developed by Hoffmann et al {Hoffmann, 2000 #79}. In brief, eight plasmids encoding each gene segment were transfected into co-cultured 293T and SIAT cells. Recovered viruses were then propagated in eggs. HK14e HA was mutated at Y159S using forward primer (CTTAAACAGCAAATACCCAGCATTGAACGTGACT) and reverse primer (TATTTGCTGTTTAAGTGGGTCAACCAATTT). RG viruses bearing wild-type and Y159S HA were sequenced and compared with native HK14e virus in HI assay using antisera raised against HK14e and Sw13e, and human mAbs generously provided by Alain Townsend (MRC Weatherall Institute of Molecular Medicine, UK). Q129C mAb was generated from B cells of a person exposed to A/Victoria/361/2011, and selects for substitutions at positions 160 and 194 in site B. RG HK14e was antigenically similar to HK14e whereas RG Y159S was distinct, and was poorly recognized by Q12C9 consistent with antigenic change within site B.

Table S8. HI titres of antisera and mAbs against wild-type and reverse genetics viruses

	Virus			
Antisera/mAb	HK14e	RG HK14e HA	RG Y159S HA	Sw13e
Anti-HK14e	**1280**	1280	320	80
Anti-Sw13e	80	80	160	**640**
Q12C9	80	80	20	20

Antigenic site positions that vary between HK14e and strains circulating before and after vaccination

Table S9

	Antigenic Site and Amino Acid Position (H3 numbering) [a]																									
	Site E					Site A						Site B						Site C						Site D			
Virus (clade)	**62**	**91**	**92**	**94**	**261**	**131**	**135**	**138**	**142**	**144**	**145**	**128**	**159**	**160**	**193**	**194**	**198**	**45**	**48**	**53**	**278**	**311**	**312**	**121**	**171**	**212**	**223**
HN09 (1)	K	S	K	H	R	T	T	A	R	K^{-}	N	T^{+}	F	K^{-}	F	L	A	S^{-}	T	D	N	Q	N	N	N	T	V
HN12 (3c1)	E	S	K	Y	R	K	T	A	R	N^{+}	S	T^{+}	F	K^{-}	F	L	S	S^{-}	T	N	N	Q	S	N	N	A	I
HN14 (3c3a)	E	S	K	Y	R	T	T	S	G	N^{+}	S	A^{-}	S	K^{-}	F	L	S	N^{+}	I	D	K	Q	S	N	N	A	I
HK14e (3c2a)	**E**	**S**	**K**	**Y**	**R**	**T**	**T**	**A**	**R**	**S**$^{-}$	**S**	**T**$^{+}$	**Y**	**K**$^{-}$	**F**	**P**[e]	**S**	**N**$^{+}$	**I**	**D**	**K**	**H**	**S**	**N**	**N**	**A**	**I**
HK14 (3c2a)	E	S	K	Y	R	T	T	A	R	S^{-}	S	T^{+}	Y	T^{+}	F	L	S	N^{+}	I	D	K	Q	S	N	N	A	I
HN17[b] (3c2a1)	E	S	K	Y	R	T	T	A	R	S^{-}	S	T^{+}	Y	T^{+}	F	L	S	N^{+}	I	D	N	H	S	K	K	A	I
HN17[c] (3c2a1b)	G	S	R	Y	R	T	K	A	G	S^{-}	S	A^{-}	Y	T^{+}	F	L	S	N^{+}	I	D	K	Q	S	K	K	A	I
HN17[d] (3c2a2)	E	S	K	H	Q	K	T	A	K	R^{-}	S	T^{+}	Y	T^{+}	F	L	S	N^{+}	I	D	K	H	S	N	N	A	I
Ka17 (3c3a)	E	N	K	Y	R	T	T	S	G	K^{-}	S	A^{-}	S	K^{-}	S	L	S	N^{+}	I	D	K	Q	S	N	N	A	I

a: residues are coloured according to amino acid properties as in Figure 2b,c

b: A/Ha Nam/EL177772/17 ~ Si16, NC16

c: A/Ha Nam/EL17804/17, A/Ha Nam/EL17805/17 ~ Br18

d: A/Ha Nam/EL17762/17, A/Ha Nam/EL17795/17~ Sw17

e: egg adaptive substitution

+/-: Amino acid substitutions that result in the gain (+) or loss (-) of a glycosylation site are coloured in pink.

Section 9: Titre increments post-vaccination in participants with and without A(H3N2)$^+$ ILI post-vaccination

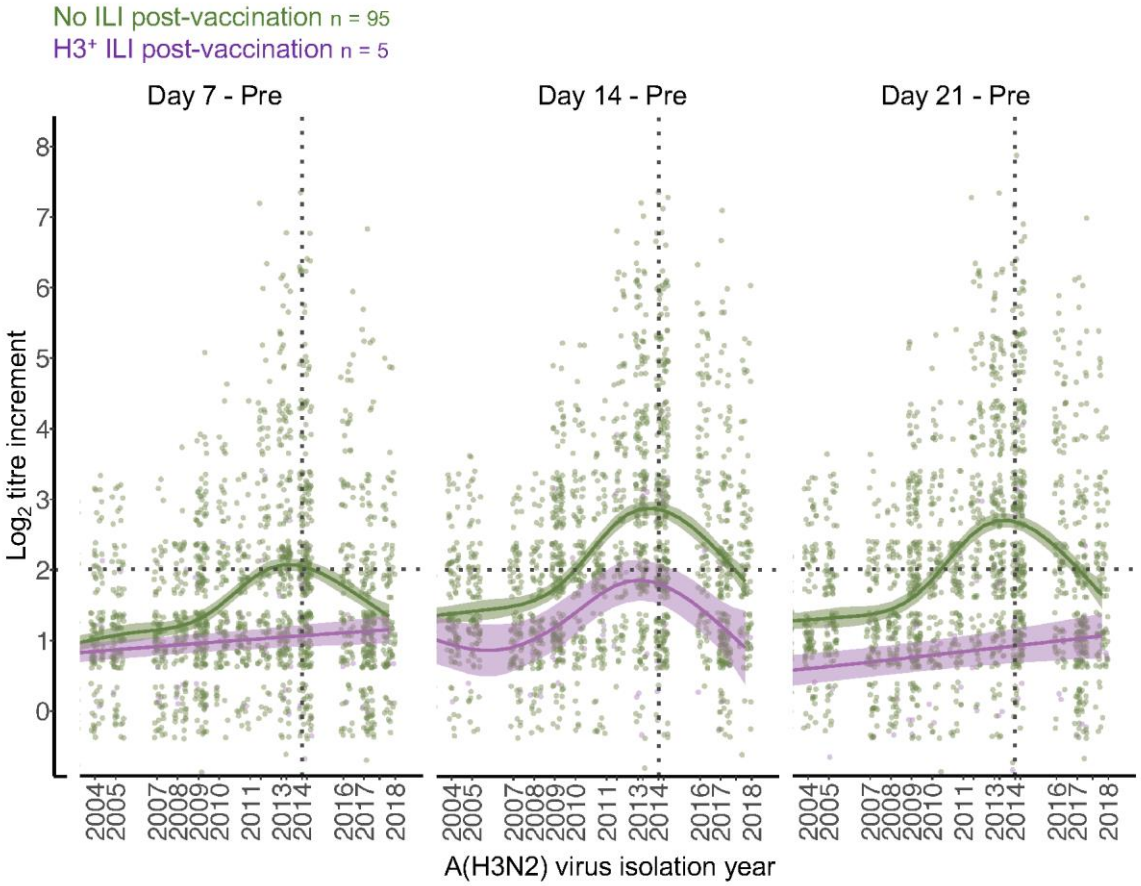


Figure S13. Delayed titre rise and poorly maintained titres post-vaccination in participants who developed ILI post-vaccination. GAM fit of $\log_2$ antibody titre increments across recent strains at various post-vaccination time points compared to baseline (lines show GMR with 95% CI) for participants who had A(H3N2)$^+$ ILI post-vaccination (purple) or who had not (green) against A(H3N2) viruses circulating since 2004.

Section 10: Post-infection landscapes of vaccinated versus unvaccinated participants

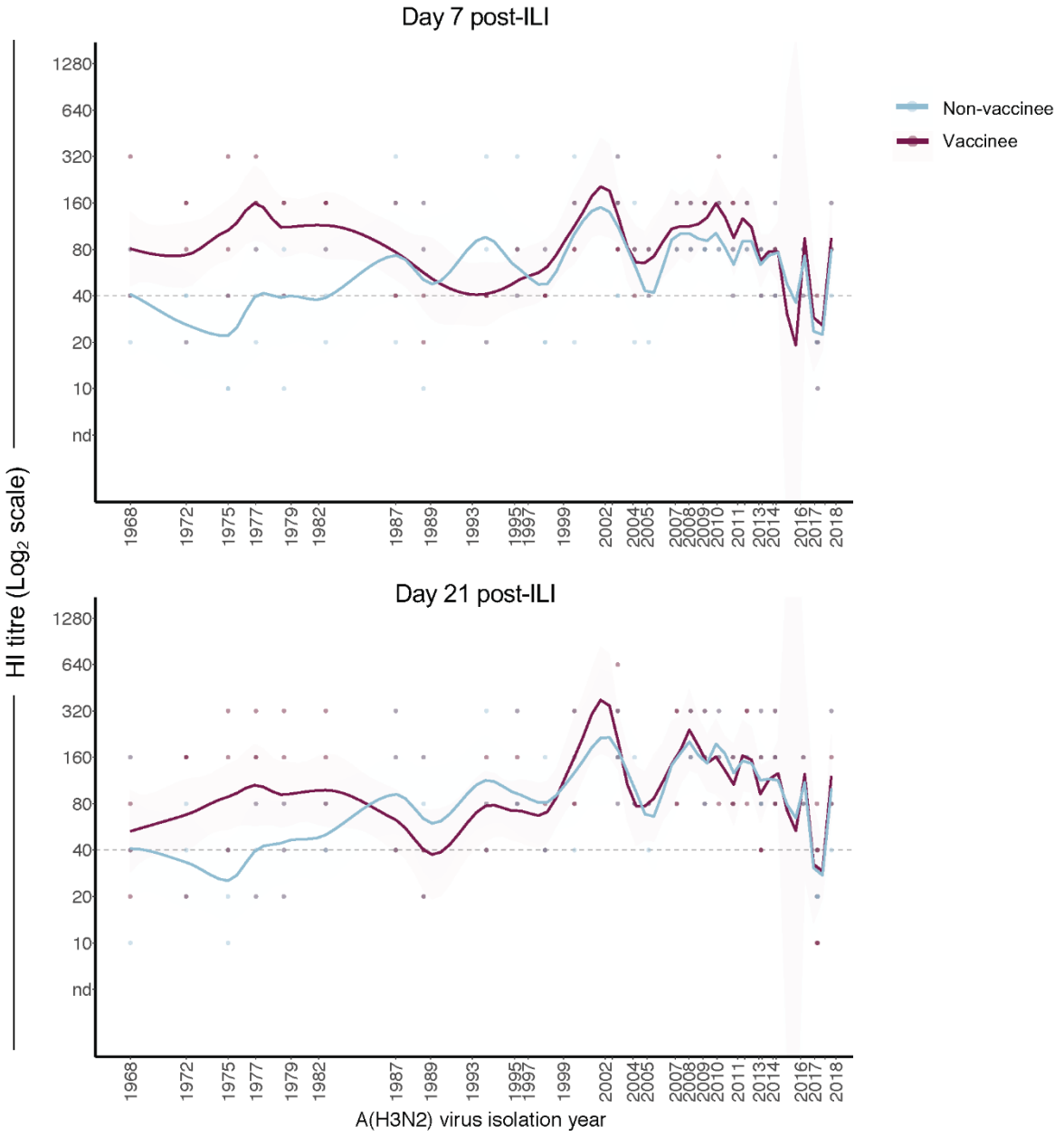


Figure S14. Antibody landscapes post-infection in vaccinated and unvaccinated participants. Lowess fit of antibody titres grouping vaccinees who developed A(H3N2)$^{+}$ ILI post-vaccination (n=5) and participants who did not receive influenza vaccination and became infected on 2017 (n=3). Lines show GMTs and dots represent individual titres.

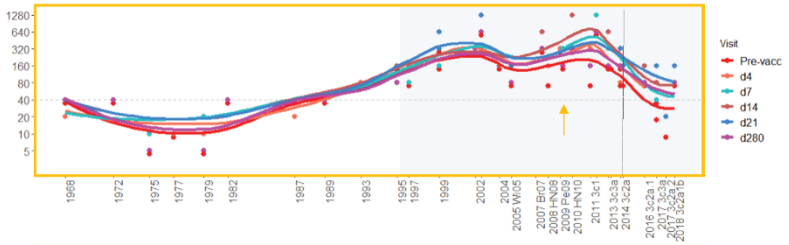
Section 11: Individual Antibody Landscapes

In this section we present titres to each strain at each time point for individual study participants (100 plots in total) arranged by year of birth from most recent. Blue shading indicates that viruses in this region circulated during that participant's lifetime. Participants who had prior infection detected as seroconversion without illness are indicated by a yellow border with yellow arrows indicating the infecting strain(s). Participants who had prior infection detected as RTPCR confirmed illness are indicated by a red border with red arrows indicating the infecting strain(s).

Notable trends include pronounced titre rises post-vaccination among the oldest participants (born before 1950, p 34), particularly if recently infected. Similarly, when comparing groups of participants born in the same year (1973 p 24; 1960 p 31, 1958 p 32), robust tire rise that remained detectable by d280 was associated with prior infection, and was largely against viruses circulating post-2000, whereas pre-vaccine titres tended to be relatively high against early life strains.

YOB

1996



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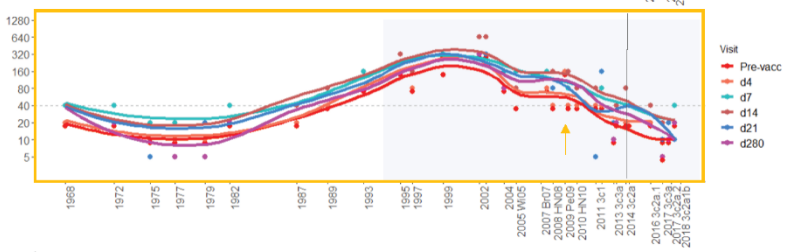

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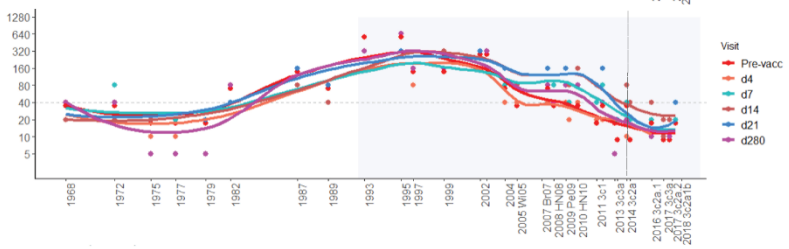

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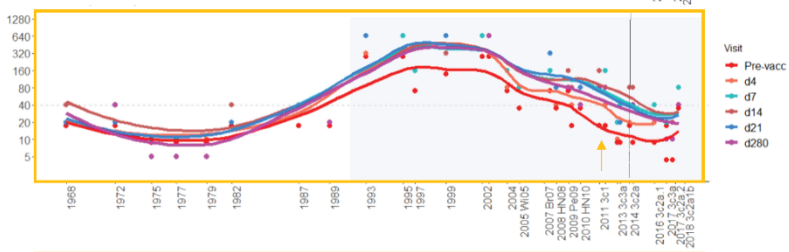

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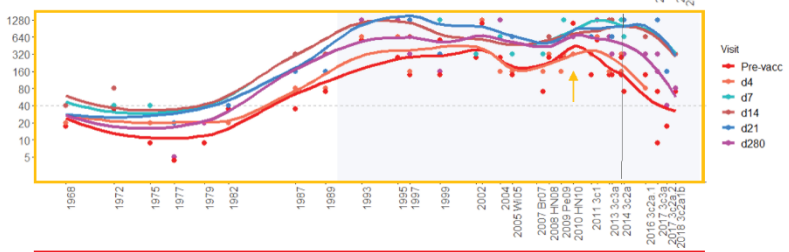

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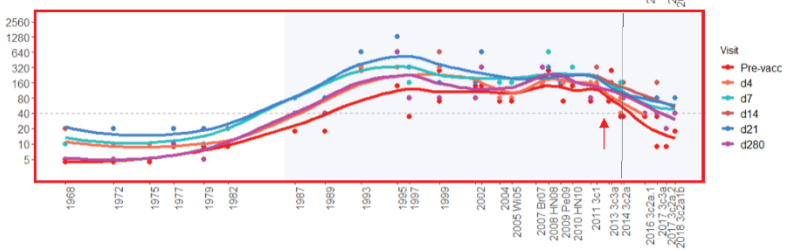

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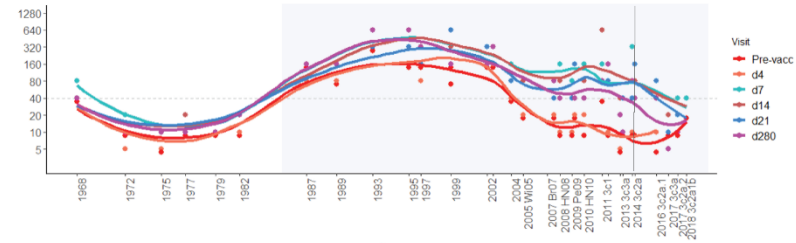

YOB

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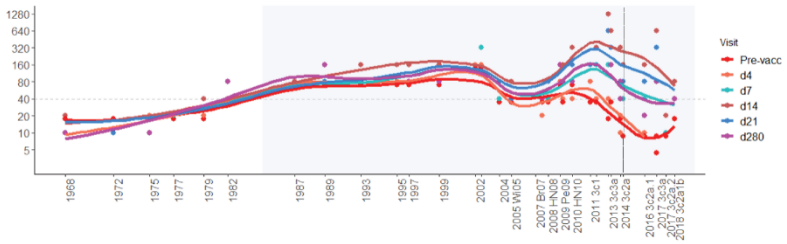

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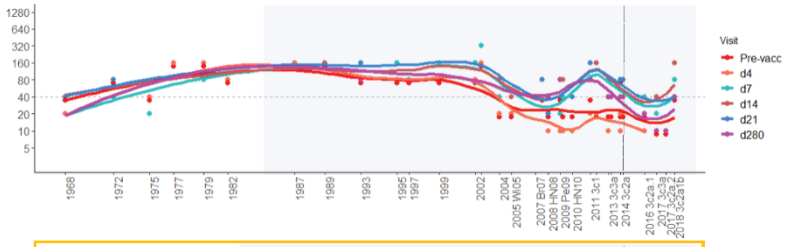

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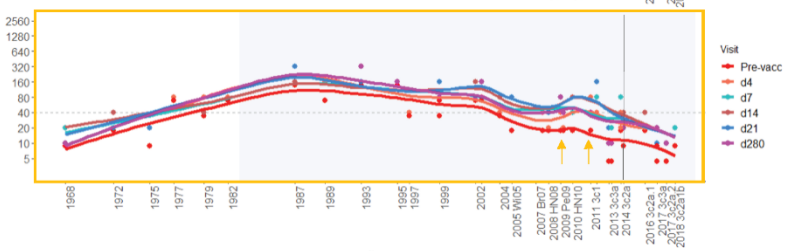

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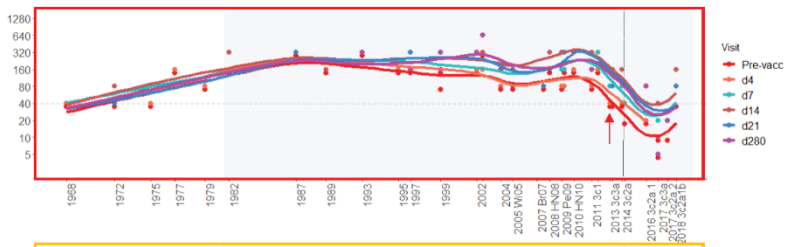

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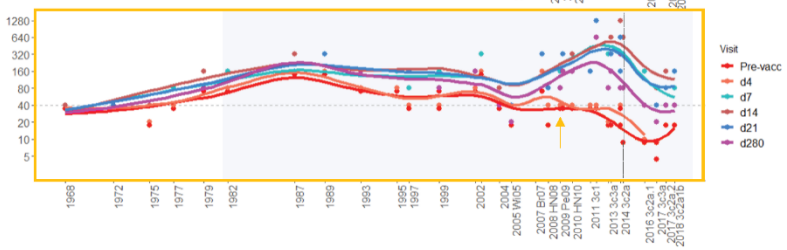

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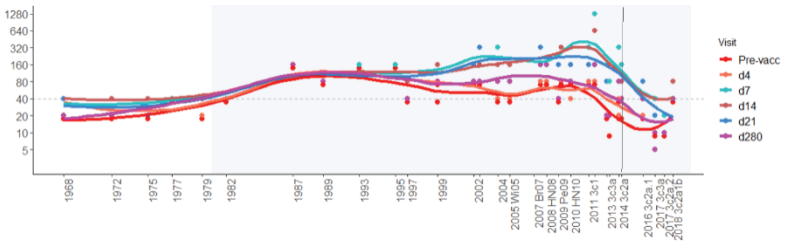

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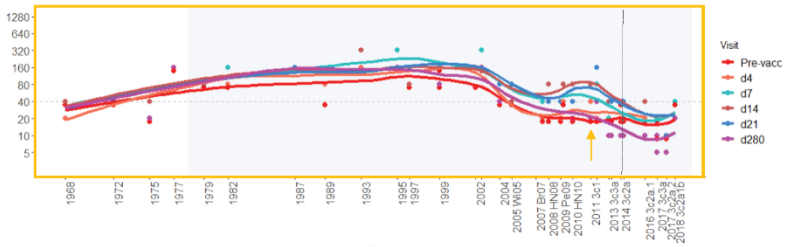
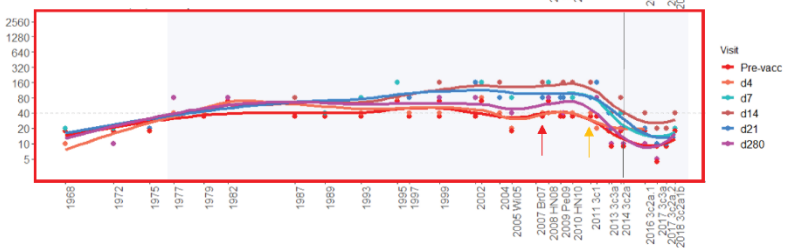


YOB

1978

1977



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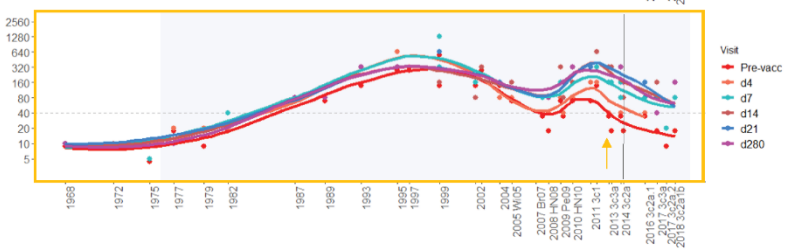

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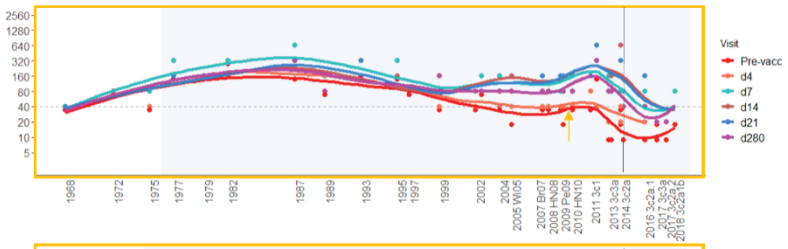

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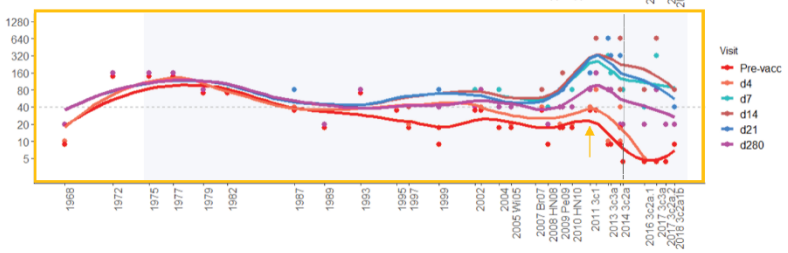

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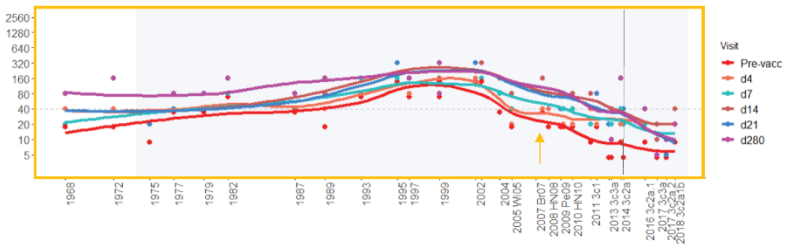

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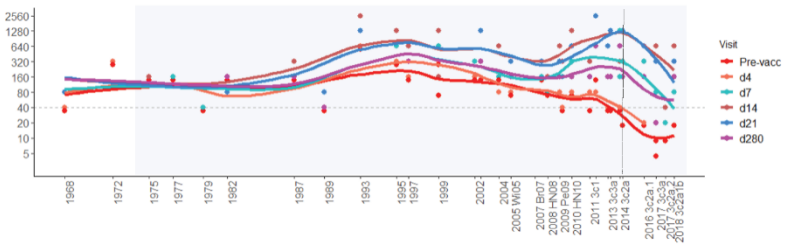
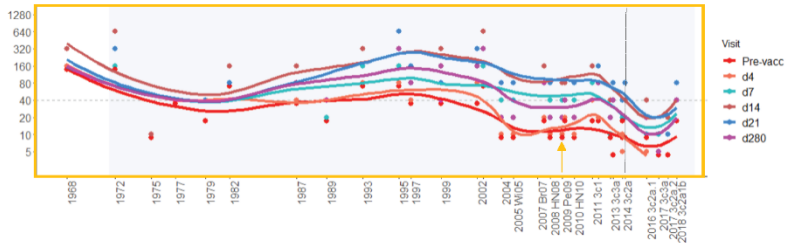
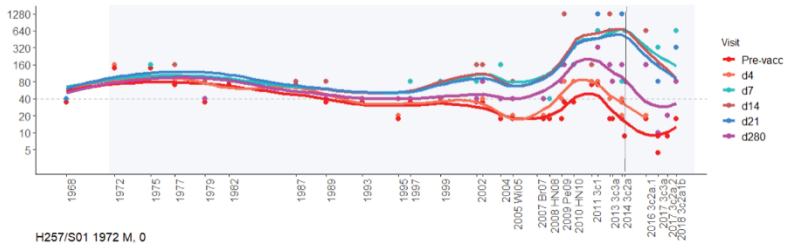
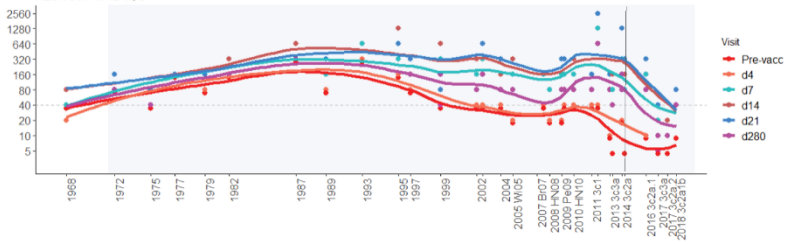


YOB

1972







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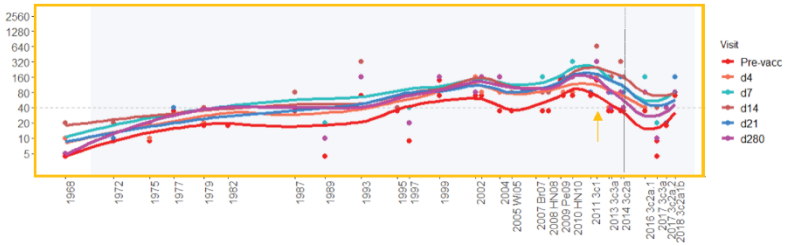

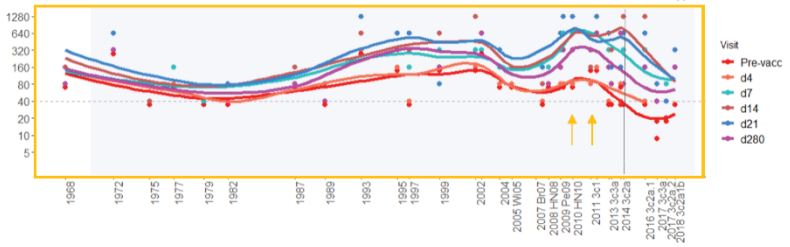

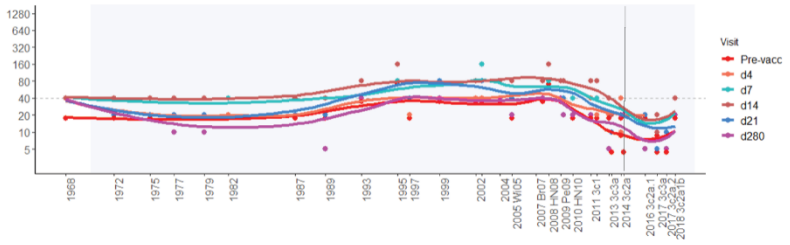

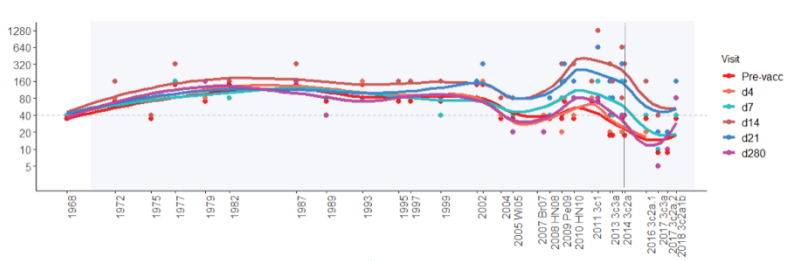

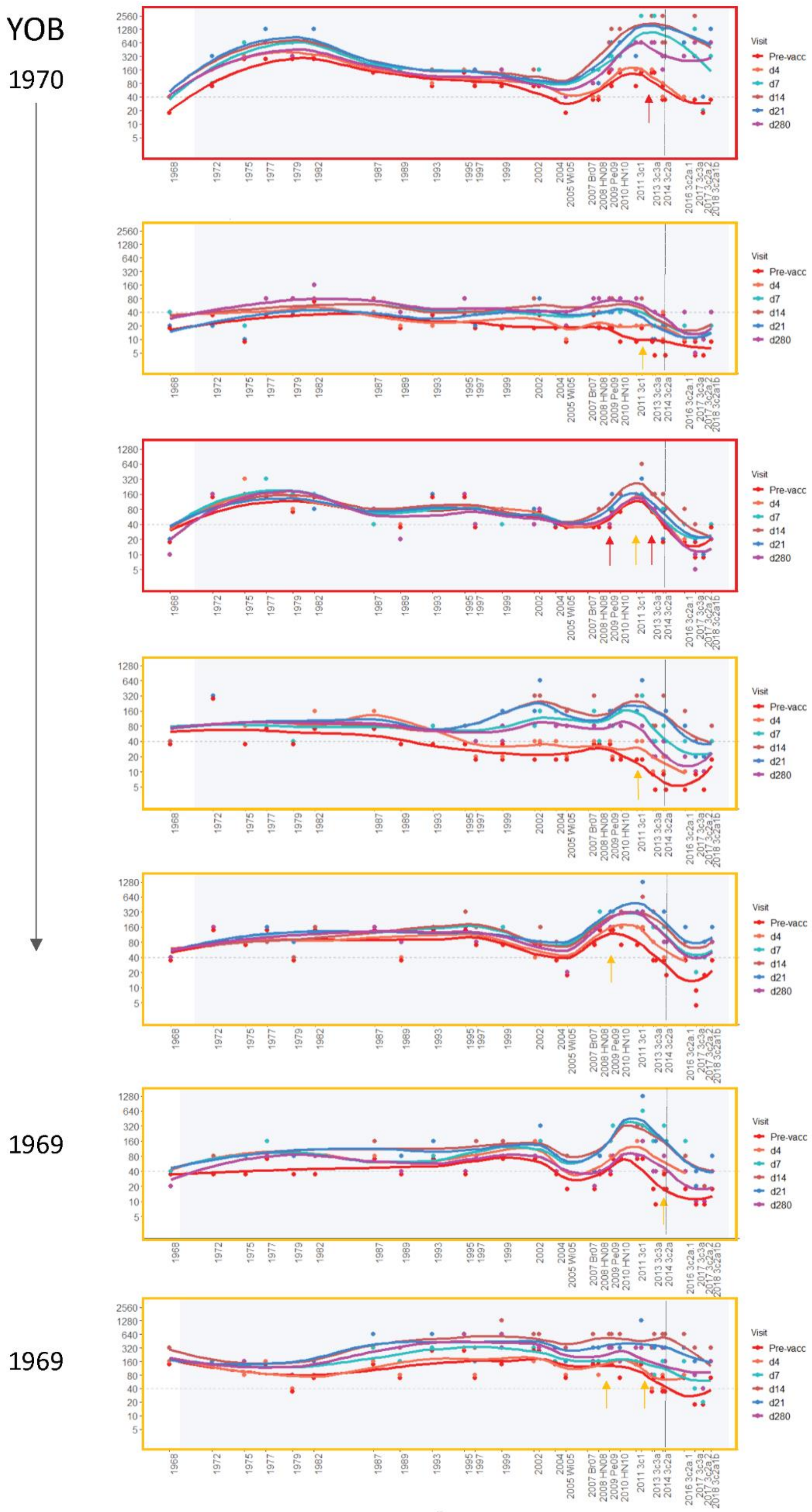
YOB
1970
1969
1969
Visit
Pre-vacc
d4
d7
d14
d21
d280
2560
1280
640
320
160
80
40
20
10
5
1968
1972
1975
1977
1979
1982
1987
1989
1993
1995
1997
1999
2002
2004
2005 WI05
2007 Br07
2008 HN08
2009 Pe09
2010 HN10
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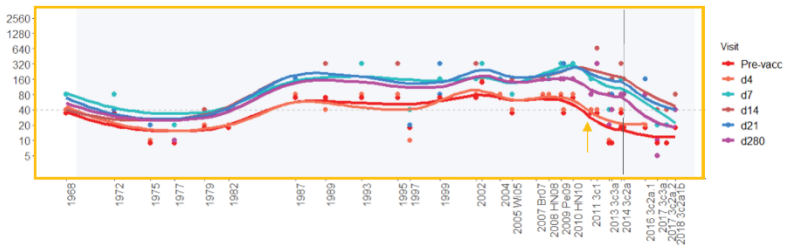
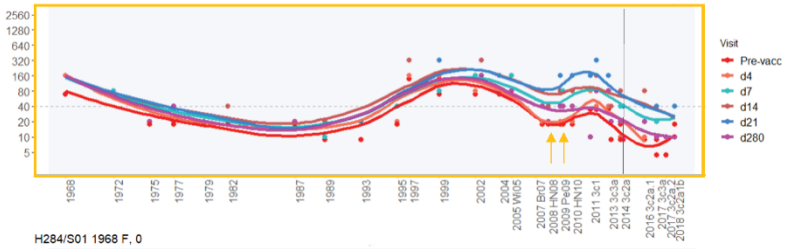
2018 3c2a1b

YOB

1969



1968



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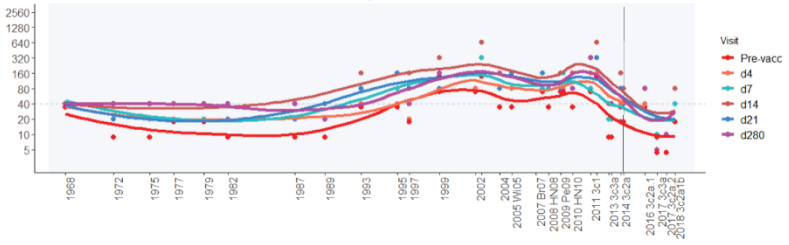

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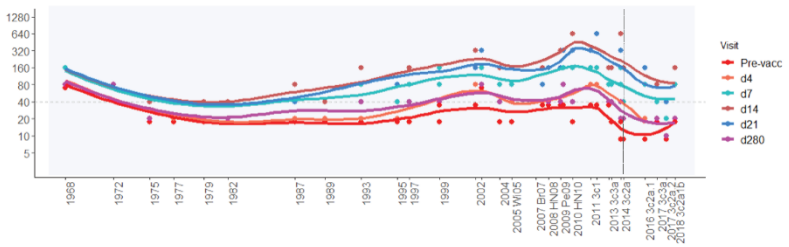
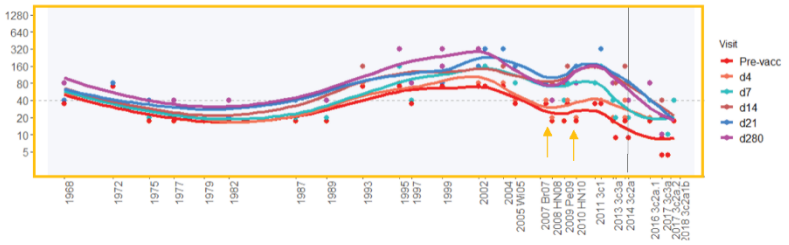
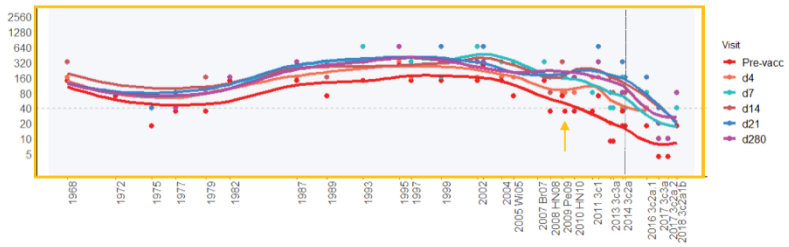
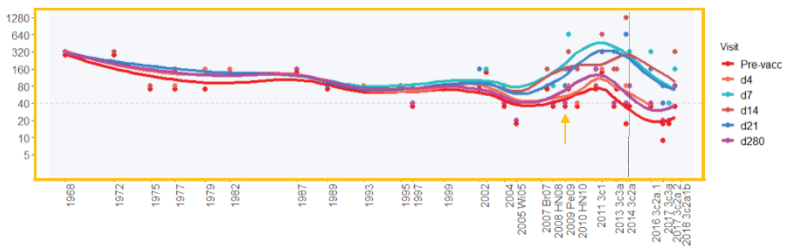


1967







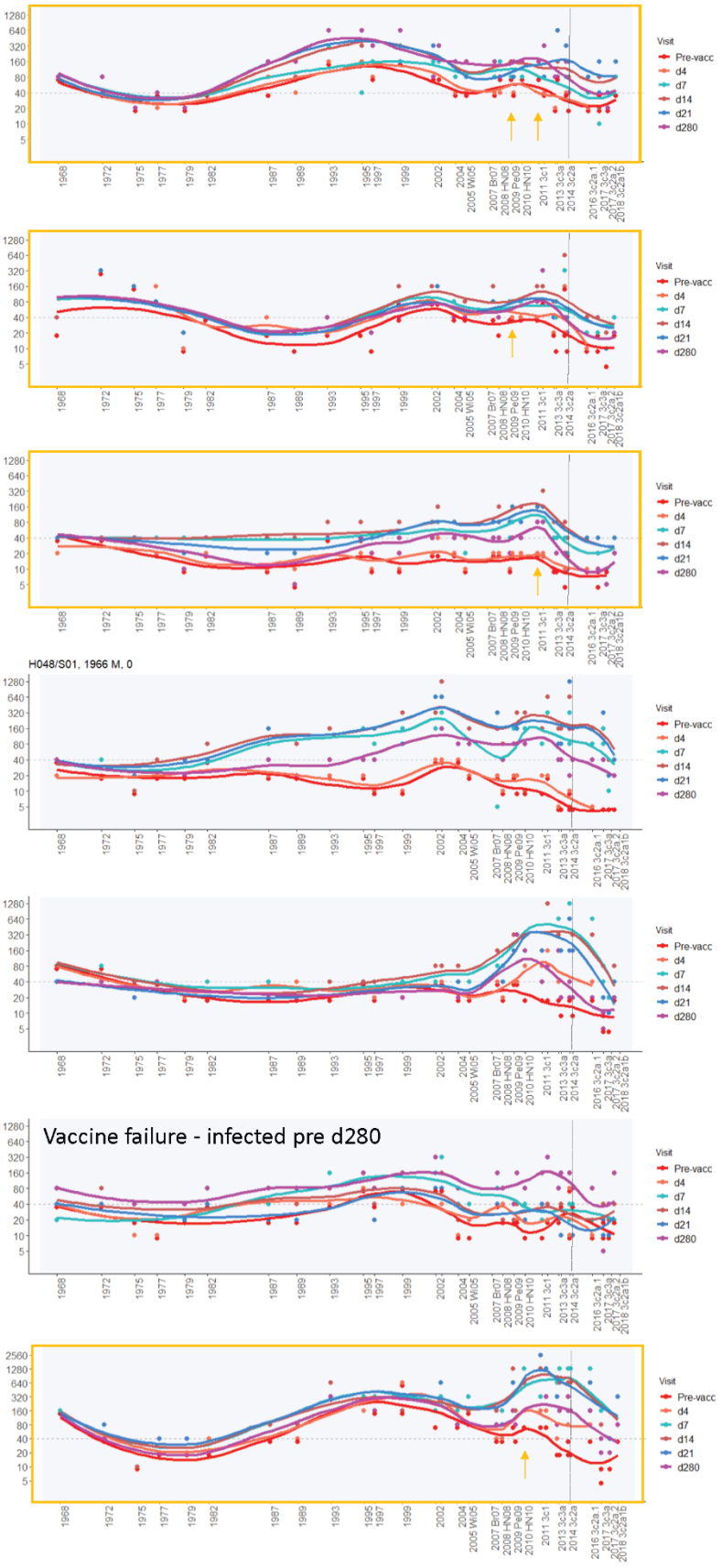
YOB

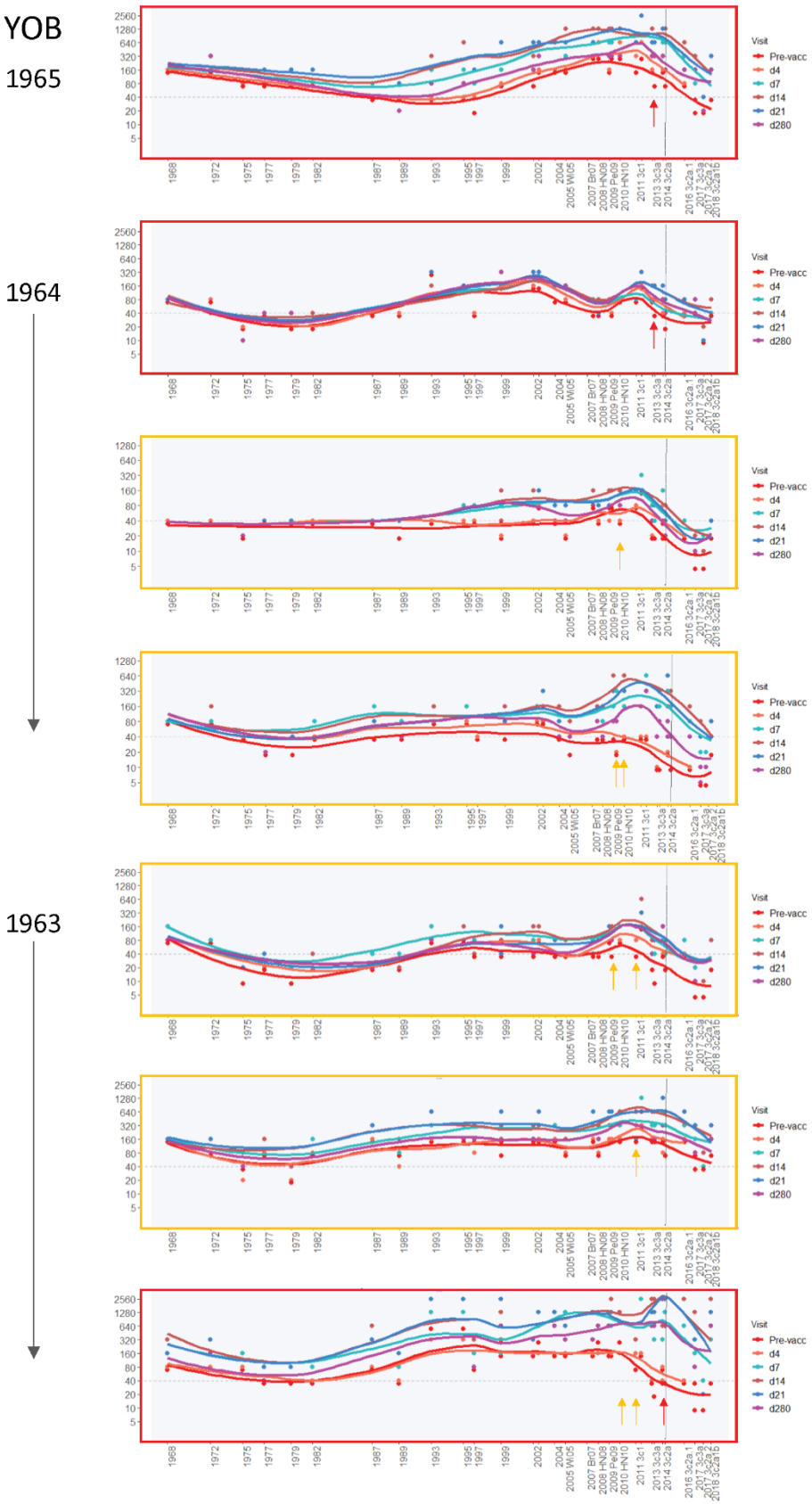
1967

1966

1965

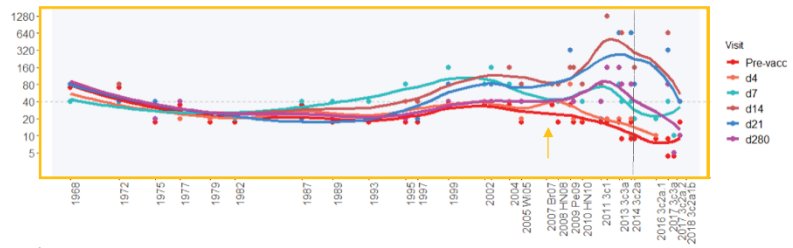
1965




YOB
1965
1964
1963
Visit
Pre-vacc
d4
d7
d14
d21
d280

YOB

1963



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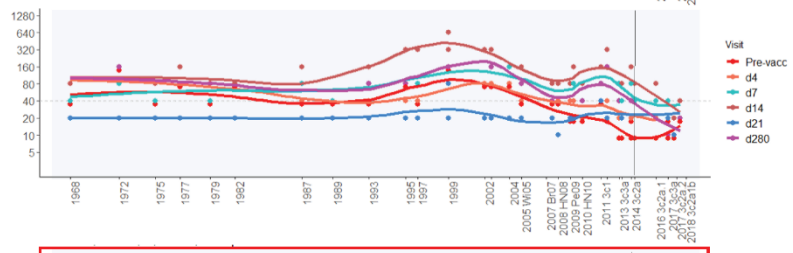

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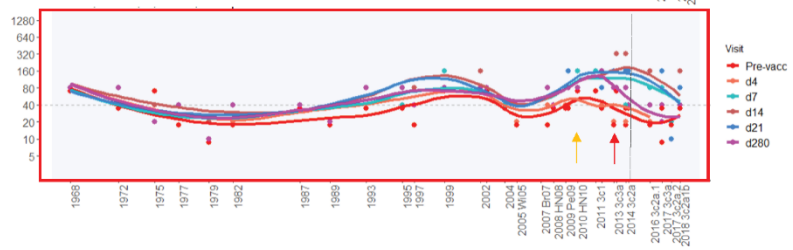
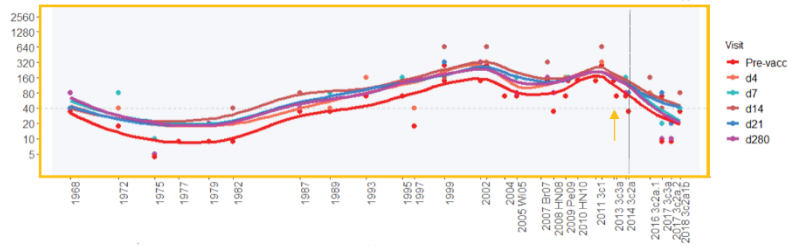
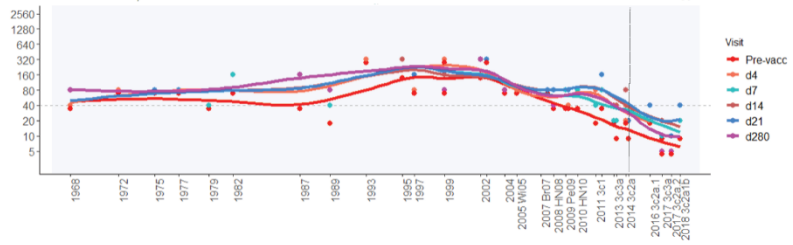
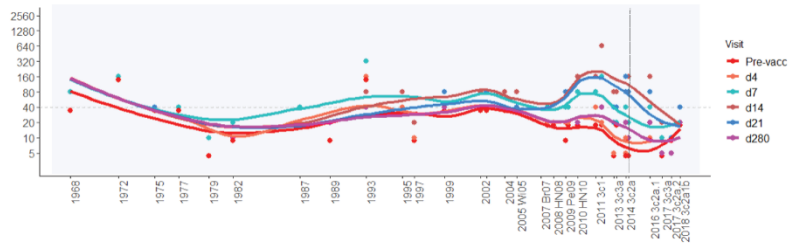


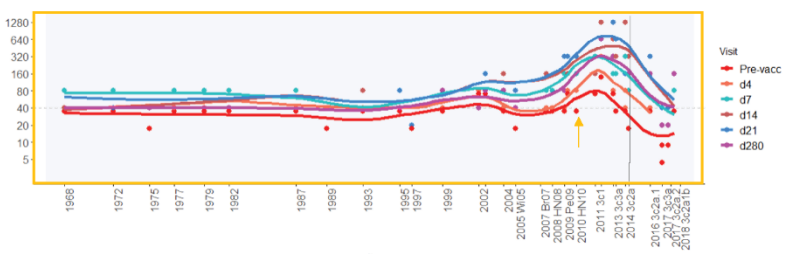




1961

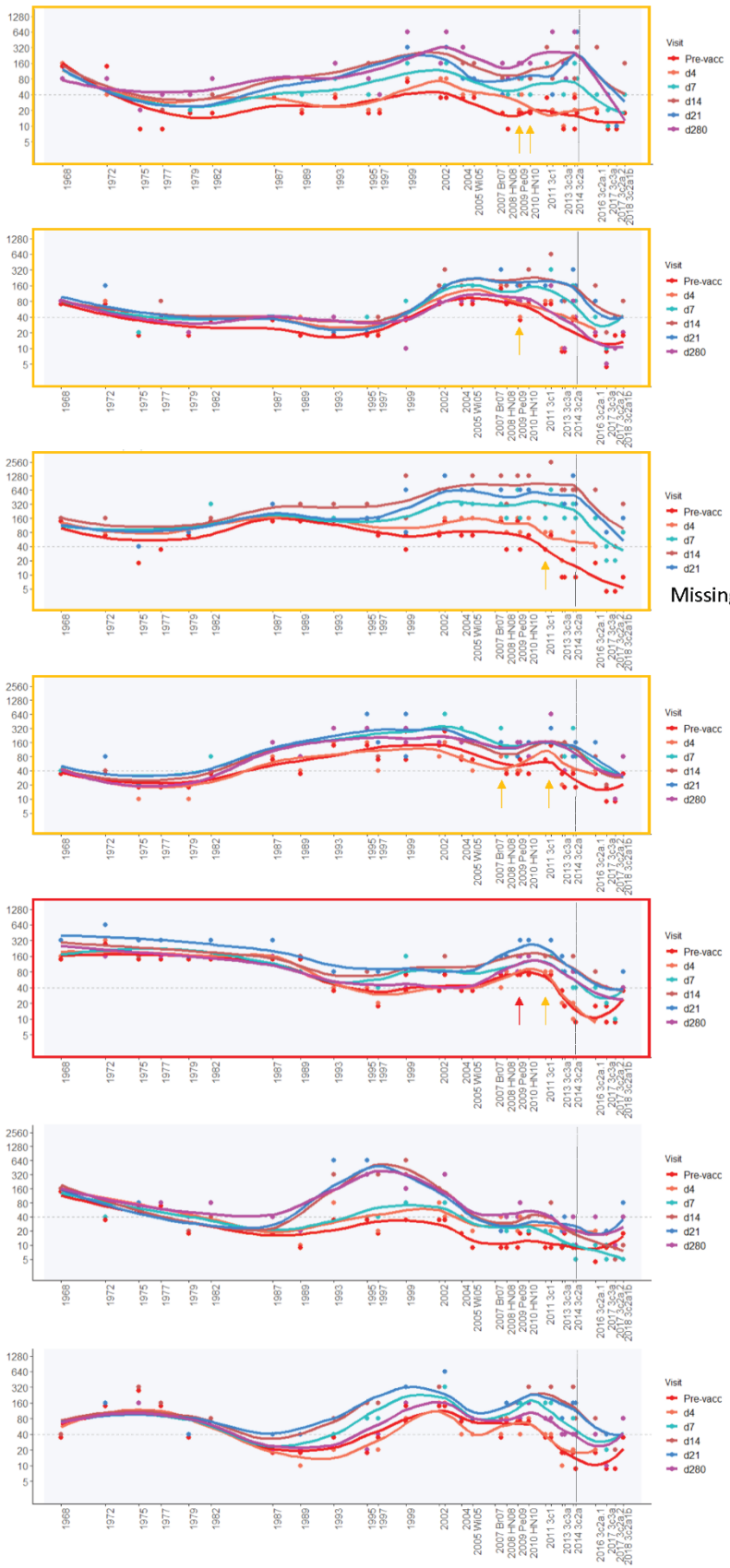


1960



YOB

1960

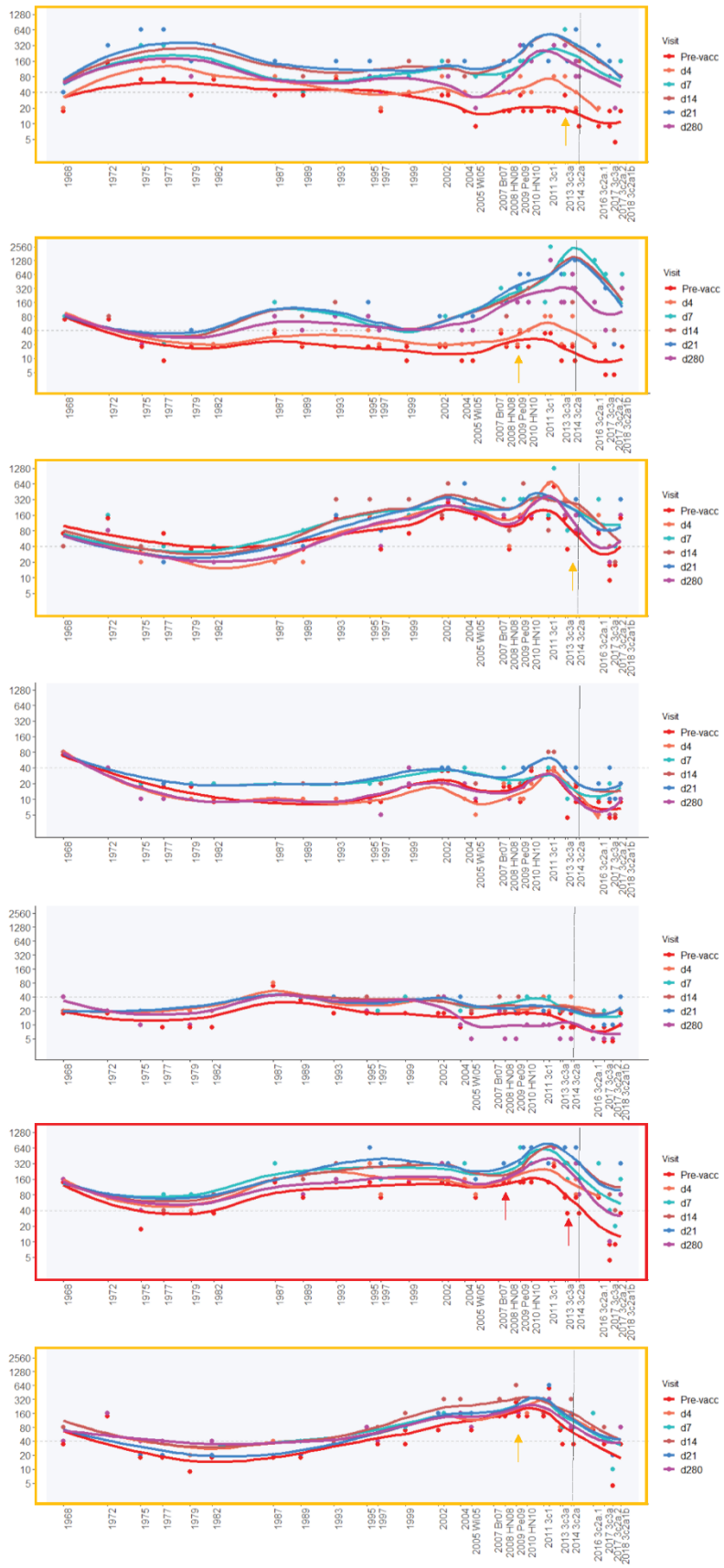


Missing d280

1959

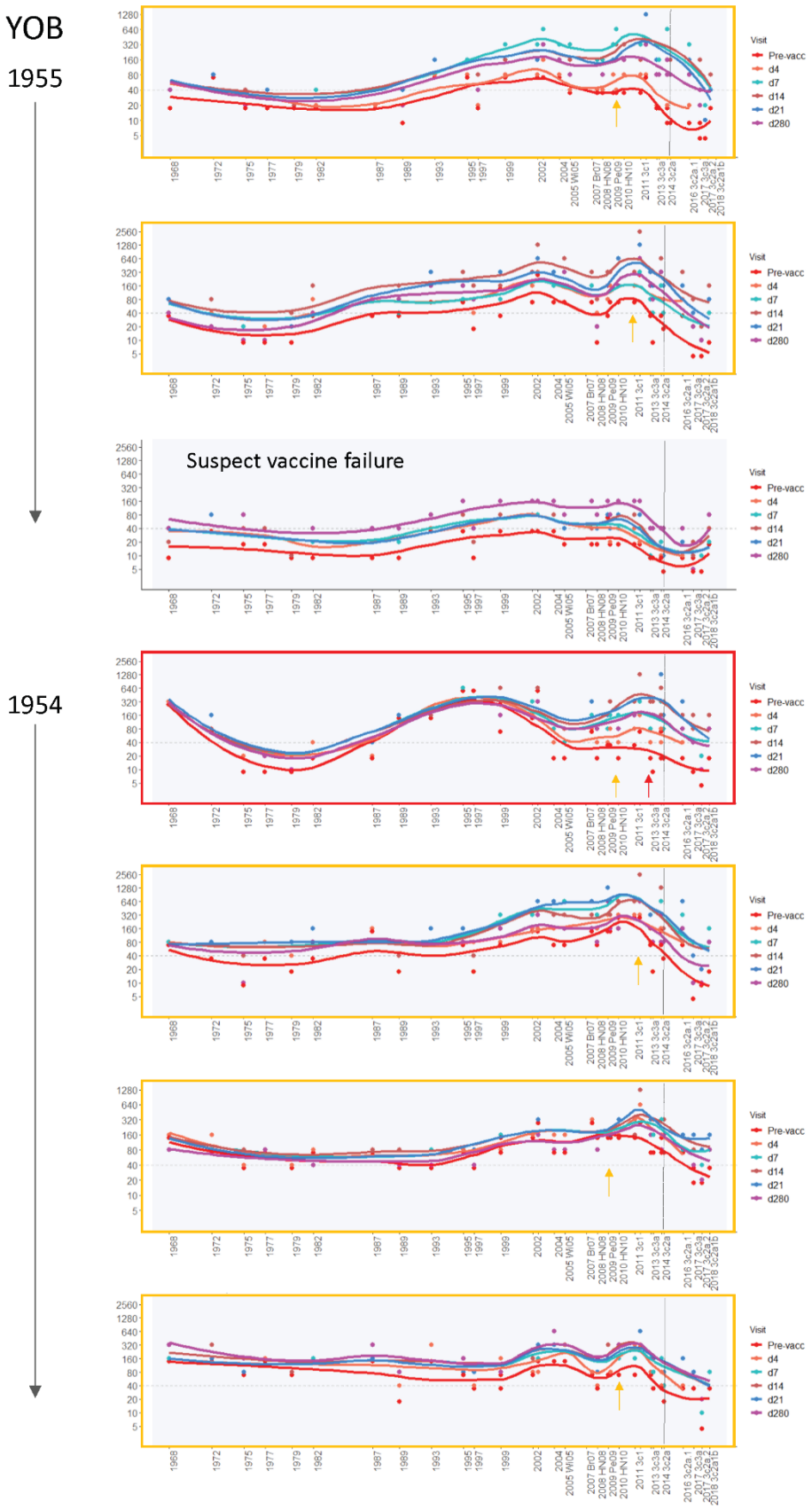
YOB

1958



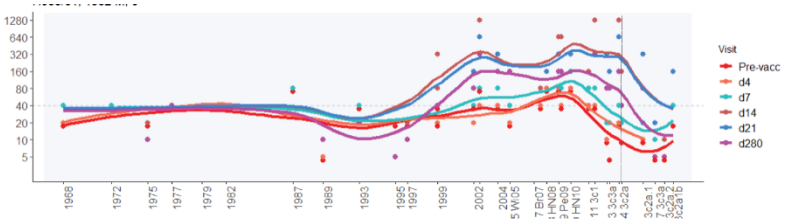
1957

1956

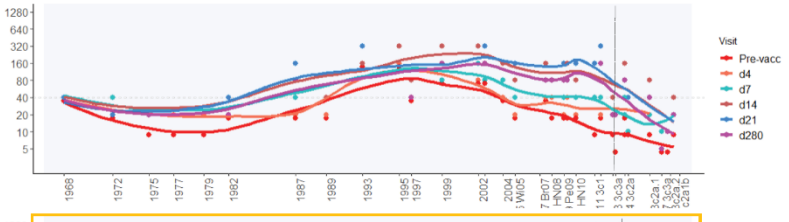

YOB
1955
1954
Suspect vaccine failure
Visit
Pre-vacc
d4
d7
d14
d21
d280

YOB

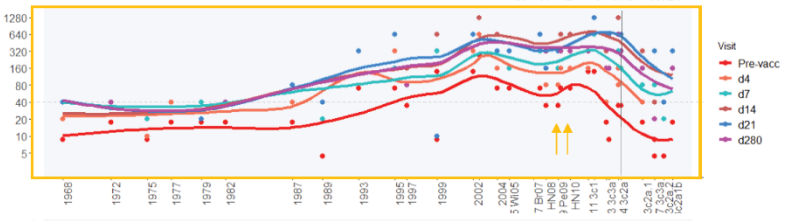
1952



1950



1947



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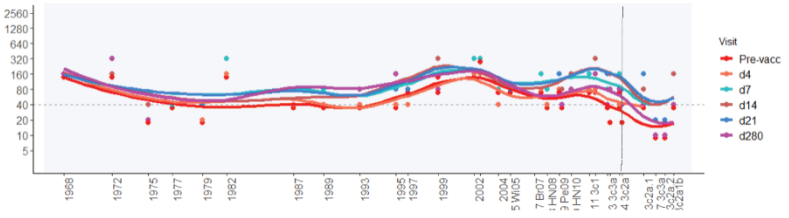

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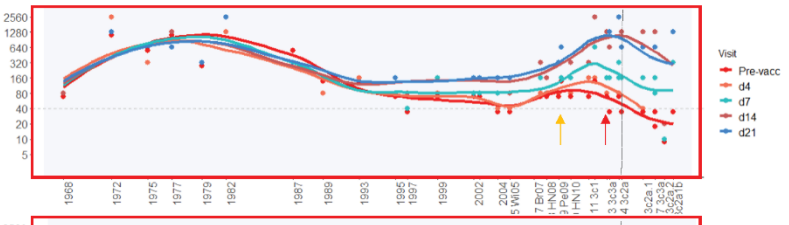

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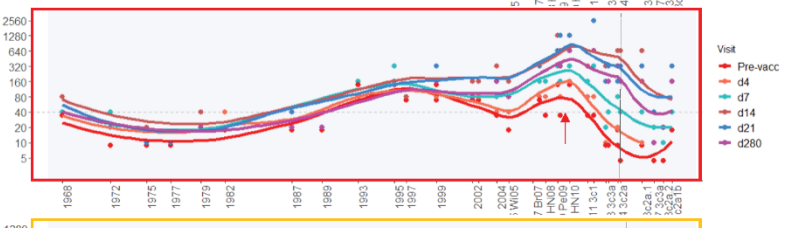
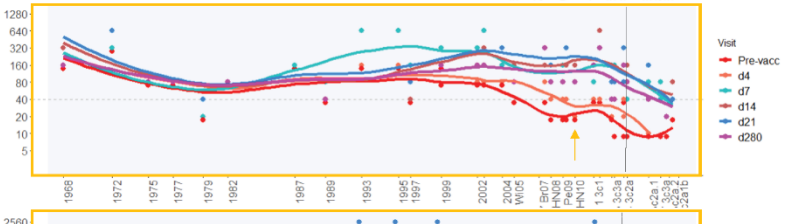
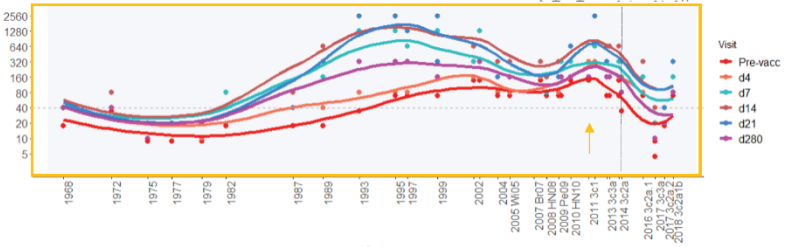


1939



1935



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1. Lin YP, Gregory V, Collins P, et al. Neuraminidase receptor binding variants of human influenza A(H3N2) viruses resulting from substitution of aspartic acid 151 in the catalytic site: a role in virus attachment? *Journal of virology* 2010; **84**(13): 6769-81.
2. Beyer WE, Palache AM, Luchters G, Nauta J, Osterhaus AD. Seroprotection rate, mean fold increase, seroconversion rate: which parameter adequately expresses seroresponse to influenza vaccination? *Virus Res* 2004; **103**(1-2): 125-32.