Viral Shedding in Recipients of Live Attenuated Influenza Vaccine in the 2016–2017 and 2017–2018 Influenza Seasons in the United Kingdom

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(See the Editorial Commentary by Monto on pages 2514–6.)

Background. The (H1N1)pdm09 live attenuated influenza vaccine (LAIV) strain was changed for the 2017–2018 influenza season to improve viral fitness, following poor protection against (H1N1)pdm09 viruses in 2015–2016. We conducted LAIV virus shedding studies to assess the effect of this change.

Methods. Children aged 2–18 years were recruited to receive LAIV in the 2016–2017 (n = 641) and 2017–2018 (n = 362) influenza seasons. Viruses from nasal swabs taken 1, 3, and 6 days postvaccination were quantified by reverse-transcription polymerase chain reaction and area under the curve titers were determined. Presence and quantity of shedding were compared between strains and seasons with adjustment for age and prior LAIV (n = 436), inactivated seasonal vaccine (n = 100), or (H1N1)pdm09 vaccine (n = 166) receipt.

Results. (H1N1)pdm09 detection (positivity) in 2016–2017 and 2017–2018 (11.2% and 3.9%, respectively) was lower than that of H3N2 (19.7% and 18.7%, respectively) and B/Victoria (28.9% and 33.9%, respectively). (H1N1)pdm09 positivity was higher in 2016–2017 than 2017–2018 (*P* = .005), but within shedding-positive participants, the (H1N1)pdm09 titer increased in 2017–2018 (*P* = .02). H3N2 and influenza B titers were similar between seasons. Positivity declined with age, and prior vaccination reduced the likelihood of shedding influenza B but not (H1N1)pdm09.

Conclusions. The (H1N1)pdm09 titer increased in 2017–2018, indicating more efficient virus replication in shedding-positive children than the 2016–2017 strain, although overall positivity was reduced. Age and vaccination history require consideration when correlating virus shedding and protection.

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Keywords. influenza; LAIV; vaccine effectiveness; virus shedding.

An intranasally administered live attenuated influenza vaccine (LAIV; FluMist/Fluenz; AstraZeneca/MedImmune) was licensed in 2003 in the United States (US), where annual vaccination of 6-month-olds to 18-year-olds has been recommended since 2008 [1]. LAIV has been preferentially recommended for pediatric vaccination in some countries, including the United Kingdom (UK), as it provides ease of administration and likely more durable and broader protection than inactivated influenza vaccines (IIVs) [2].

However the subsequent performance of LAIV in the field has been variable globally. In the 2013–2014 influenza season, vaccine effectiveness (VE) in the US was significantly lower for LAIV than IIV, particularly against (H1N1)pdm09 viruses [3, 4]. For the 2015–2016 influenza season, the A/California/07/09(H1N1)pdm09-like strain was replaced with A/Bolivia/559/2013-like virus in a quadrivalent formulation including both B/Victoria (B/Vic)– and B/Yamagata (B/Yam)–lineage viruses [2]. However, no statistically significant VE against (H1N1)pdm09 was observed by the US Centers for Disease Control and Prevention in 2015–2016 [5, 6]. The Advisory Committee on Immunization Practices subsequently recommended temporary suspension of LAIV use in 2016–2017 in the US [7].

LAIV has been licensed for pediatric vaccination in Europe since 2010 and was first included in the UK vaccine schedule in 2013 as the vaccine of choice for 2- to 16-year-olds [2]; however, IIV continues to be recommended for high-risk children aged 6–24 months for whom LAIV is not licensed [8]. In contrast to the US, surveillance data from the UK, Finland, and Canada in

2015–2016 demonstrated (H1N1)pdm09 VE estimates comparable to previous seasons and markedly higher than that observed in the US [8–11]. Several potential causes have been postulated to explain the low (H1N1)pdm09 VE estimates observed in the US, including vaccine–virus interference, prior vaccination, replicative fitness of (H1N1)pdm09, or biases arising from differences in design between the various observational studies [11–13]. As low VE was observed with both trivalent [14–16] and quadrivalent LAIV [5, 6] virus interference specific to the quadrivalent formulation is considered less likely [17]. However, viral replicative fitness is a potential factor as both the recently mammalian adapted A/California/07/09(H1N1)pdm09-like and A/Bolivia/559/2013-like strains have shown reduced replication in human alveolar and primary nasal epithelial cells, compared to previous H1N1 strains that have shown high effectiveness in children [12]. Consequently for the 2017–2018 vaccine, the A/Bolivia/559/2013-like virus was replaced with A/Slovenia/2903/15-like virus, shown to have increased replicative ability and higher seroconversion rates in children aged 2 to <4 years [18, 19].

Although LAIV was not recommended for use in the US in 2016–2017 [7, 18], it was used in Europe, including the UK, and Canada [2, 20]. The UK Joint Committee on Vaccination and Immunisation (JCVI) reviewed UK data and recommended continued LAIV use in 2016–2017, with close monitoring of performance [2]. In the UK, the overall VE of LAIV against all subtypes in 2016–2017 was 65.8% (95% confidence interval [CI], 30.3%–83.2%) (Supplementary Table 1), in a season dominated by H3N2 and influenza B; however, due to lack of circulation, estimating (H1N1)pdm09-specific VE was not possible [21]. Although in the UK the overall VE of LAIV against all subtypes in 2017–2018 was only 26.9% (95% CI, −32.6% to 59.7%), (H1N1)pdm09-specific VE was 90.3% (95% CI, 16.4%–98.9%), suggesting that the (H1N1)pdm09 strain change may have improved replicative fitness [22]. The strain change provided an opportunity to test the hypothesis that reduced viral fitness of the (H1N1)pdm09 vaccine strain may have contributed to low VE in 2015–2016. This study was designed to quantify levels of all 4 LAIV viruses shed postvaccination from UK pediatric LAIV recipients with varying vaccination histories from cohorts recruited in 2016–2017 and 2017–2018, to determine whether a difference could be observed in viral replication and subsequent immune protection after the (H1N1)pdm09 strain change and to explore the contribution of age and prior vaccination to viral shedding.

MATERIALS AND METHODS

Study Approval

This study was approved by the UK Medicines and Healthcare Products Regulatory Agency (EudraCT numbers 2013-003592-35, 2016-002352-24, and 2017-000952-24) and the National Health Service (NHS) Human Research Authority (HRA) (approval numbers 14/LO/0227, 16/WM/0276, and 17/LO/0719)

and registered prospectively with ClinicalTrials.gov (identifiers NCT02143882, NCT02866942, and NCT03104790).

Study Design

This study was designed (1) to determine, in children with the same vaccination history, effects of the 2017–2018 (H1N1)pdm09 vaccine strain change on viral shedding and (2) to determine any association between virus shedding and prior vaccine status. This analysis utilized samples from 3 open-label, nonrandomized phase 4 studies over the 2016–2017 and 2017–2018 influenza seasons, to quantify viral shedding from LAIV recipients. Participants received LAIV according to the summary of product characteristics (SmPC) in line with UK Department of Health guidance. Nasal swabs were taken as described below.

Samples were included from 2 cohorts who received LAIV in 2016–2017 and 2017–2018. Vaccine-naive and twice LAIV-vaccinated children between the ages of 6 and <14 years at enrollment were recruited from year 3 of a longitudinal study (LAIV-Immuno; EudraCT 2013-003592-35; HRA approval number 14/LO/0227) to document serological responses to annual LAIV vaccination [23]. Inclusion/exclusion criteria were as per the approved SmPC. Recruitment was boosted with participants from a prospective, multicenter UK trial assessing LAIV safety in children with asthma (the "Safety of nasal influenza immunisation in children with asthma" study; SNIFFLE-4; EudraCT 2016-002352-24; HRA approval number 16/WM/0276), resulting in opportunistic recruitment of participants aged 2–18 years. Inclusion criteria required a physician diagnosis of asthma or recurrent wheezing, with exclusion criteria (with the exception of severe asthma) as per the SmPC and Public Health England guidance [24]. A third cohort was established for 2017–2018 (Flu-Shed study; EudraCT 2017-000952-24; HRA approval number 17/LO/0719; ClinicalTrials.gov identifier NCT02866942), in which children aged 6 to <14 years at enrollment with either LAIV vaccination in at least 2 of the 3 previous years or who had never received any influenza vaccine were recruited in 3 sites (Hertfordshire and Gloucestershire primary care sites; Imperial College Healthcare NHS Trust, London). Participant vaccination history was determined by reference to medical records and parental questionnaire. For all studies, written informed consent was given by parent/guardian as well as assent from the child. Vaccination was deferred if a participant had a fever ≥38°C on the appointment day or recent wheezing. Participating families were asked to report any adverse events requiring medical attention.

Vaccine

LAIV (Fluenz Tetra, AstraZeneca UK Ltd) was administered according to the approved SmPC. The 2016–2017 vaccine contained A/Bolivia/559/2013(H1N1)pdm09-like, A/New Caledonia/71/2014(H3N2)-like, B/Phuket/3073/2013-like (B/Yam), and B/Brisbane/60/2008-like (B/Vic) viruses. In the

2017–2018 vaccine, A/Bolivia/559/2013(H1N1)pdm09-like virus was replaced with A/Slovenia/2903/2015(H1N1)pdm09-like virus.

Sample Collection and Processing

Day of vaccination was day 0. Nasal swabs taken on days 1, 3, and 6 postvaccination were placed in 2 mL of viral transport media and either posted the same day or stored at 4°C until the next available post. Upon receipt, reverse-transcription quantitative polymerase chain reaction (RT-qPCR) samples were prepared by adding 150 µL of sample to 50 µL of lysis buffer (MagNa Pure LC Total Nucleic Acid Isolation Kit, Roche) containing internal control RNA (soil-borne cereal mosaic viral RNA) and stored at −80°C prior to testing.

Blood samples were taken prevaccination and 21 days later (window of 21–42 days). Samples were analyzed for serum antibody titers against the 4 LAIV strains by hemagglutination inhibition and neuraminidase inhibition enzyme-linked lectin assays as previously described [23].

Nucleic Acid Extraction and RT-qPCR

Viral RNA was extracted from samples, standards, and controls using a MagNAPure nucleic acid isolation system (Roche). Two quantitative multiplex 1-step RT-qPCR assays were developed to amplify specific regions of the hemagglutinin gene of all 4 LAIV viruses and the internal control RNA. A triplex assay was designed to amplify (H1N1)pdm09, H3N2, and B/Vic-lineage RNA, with the B/Yam-lineage and internal control RNA amplified in a duplex assay. RT-qPCR reactions were performed using TaqPath 1-Step Multiplex Mastermix (without ROX, with MUSTANG PURPLE) according to the manufacturer's instructions and gene-specific primers/probes (Supplementary Table 2). Amplification and analysis were performed on a QuantStudio 7-Flex platform (Applied Biosystems).

The RT-qPCR limit of detection (LOD) corresponding to 95% positivity was determined for all strains by probit analysis. Test sample viral quantities were determined from standard curves of plaque-forming units (PFU) vs cycle threshold value generated from serial 10-fold dilutions of the 4 viruses at known titers, in quadruplicate on each PCR plate. Samples were analyzed in duplicate and mean PFU values determined. If a mean PFU value fell below the LOD, the sample was reanalyzed for all 4 viruses. PFU values above the LOD were converted to PFU/mL titers.

Statistical Analyses

PFU results were log-transformed, and the area under the curve (AUC) was calculated and divided by 6 to obtain an average per day, antilogged, and multiplied by 133.33 to obtain PFU/mL/day. For day 6 swabs taken on day 5 or days 7–10, the day 6 value was estimated by linear interpolation. AUC was expressed as the geometric mean with 95% CI. Effects of age, season, and prior vaccination on shedding rates were assessed by multiple logistic regression. Linear regression on logged AUC was used to obtain age and fold differences between years after adjusting for age and vaccine history. AUC was compared using the nonparametric Kruskal-Wallis test. For season comparisons, results from 2- to 6-year-olds in both seasons were excluded due to insufficient numbers in this age group in 2017–2018. Shedding between strains within each year was compared using McNemar exact test. The target sample size for 2017–2018 was 400 which, when compared with the known sample size of >400 for 2016–2017, allowed differences in shedding rates of 10% to be detectable at 80% power and 5% significance. Serum antibody geometric mean titers were calculated with 95% CI and compared by normal-error regression. The proportion of samples displaying a minimum 4-fold increase in neutralizing antibody levels pre- to postvaccination (seroconversion) were compared by Fisher exact test. Significance was taken at 5% except for comparison of shedding rates between strains, where a *P* value of .008 was used to correct for the 6 comparisons.

RESULTS

Participants

Six hundred forty-one and 362 participants were recruited and vaccinated during the 2016–2017 and/or 2017–2018 influenza seasons, respectively; of these, 436 and 310, respectively, provided all 3 nasal swabs (Figure 1). At least 1 swab was provided by 531 participants in 2016–2017 (with 116 also providing paired pre- and postvaccination blood samples) and 354 in 2017–2018. Characteristics of the 2 cohorts were broadly similar, with some differences in age distribution (Supplementary Table 3) and prior LAIV receipt, reflecting the opportunistic recruitment of the 2016–2017 participants (Table 1). Recipients of prior trivalent inactivated influenza vaccine (TIV) were predominantly SNIFFLE-4 recruits in 2016–2017. The proportion having received the AS03 adjuvanted (H1N1)pdm09 vaccine (Pandemrix) in 2009–2010 was similar in both cohorts.

Quantitation of Viral Shedding

Samples from participants that provided 3 swabs were analyzed by RT-qPCR. (H1N1)pdm09 virus (H1) positivity was significantly lower than all other strains (*P* < .001) in both seasons. H1 virus shedding was detected in 11.2% of individuals in 2016–2017, which declined to 3.9% in 2017–2018 (*P* = .005). In both seasons, H3N2 virus (H3) positivity was similar (19.7% and 18.7% in 2016–2017 and 2017–2018, respectively), but significantly lower than the influenza B viruses (*P* < .001). B/Vic-lineage and B/Yam-lineage viruses were present in 27.5%–33.9% of samples (Table 2). Although positivity was higher for B/Vic than B/Yam, this was not significant (*P* = .49).

In individuals with detectable virus shedding, the H1 average titer (204 PFU/mL/day) was lower than other viruses in
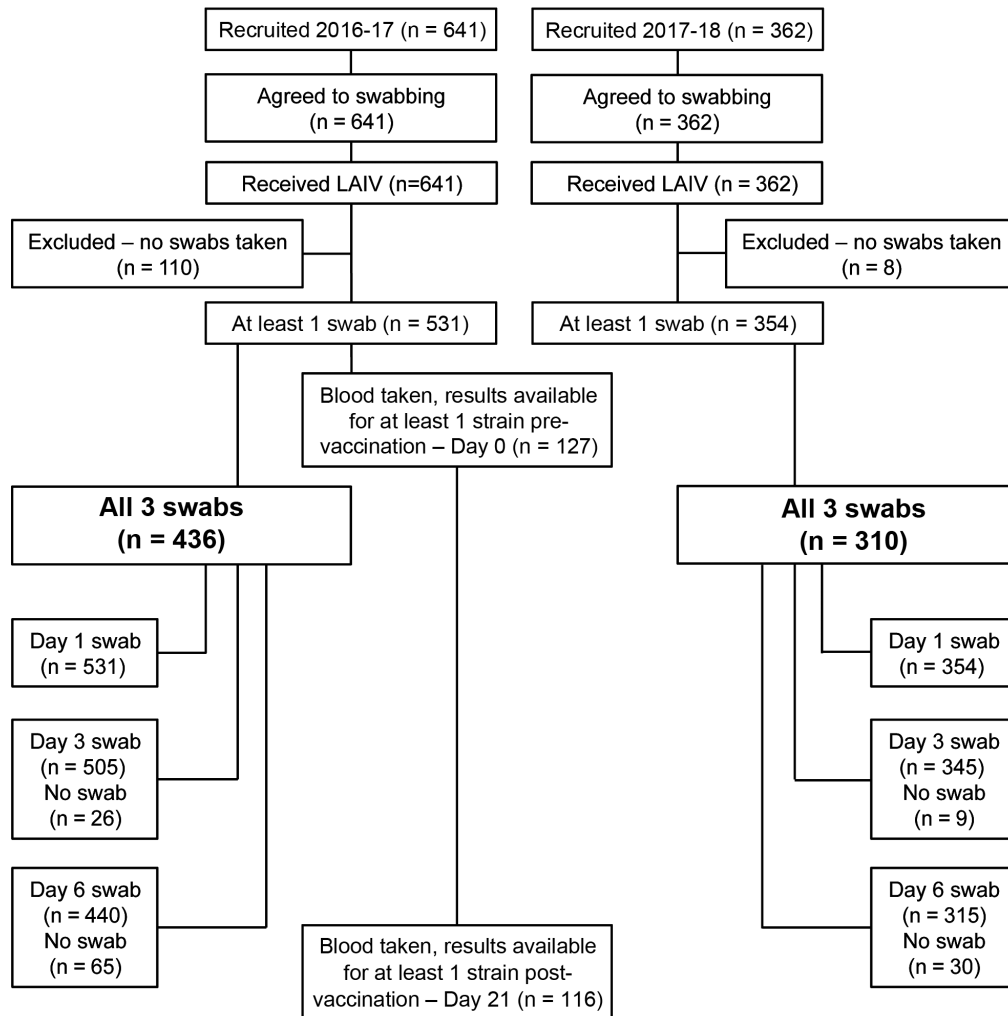
Figure 1. Study flowchart. Children aged 2–18 years, either influenza vaccine naive or with live attenuated influenza vaccination in at least 2 of the 3 previous years, were recruited during the 2016–2017 and 2017–2018 influenza seasons. Samples from participants from whom 3 swabs were received (taken on days 1, 3, and 6 postvaccination) were subjected to quantitative viral shedding analysis. Blood samples were only taken during the 2016–2017 season and used to determine serum antibody titers pre- and postvaccination. Abbreviation: LAIV, live attenuated influenza vaccination.

Table 1. Study Demographics of Participants

	Influenza Season	
Factor	2016–2017	2017–2018
Sex, male/total	241/436 (55)	144/310 (46)
Age group, y		
2–6	93 (21)	8 (3)
7–11	225 (52)	205 (66)
12–18	118 (27)	97 (31)
LAIV received in previous season, yes/total	307/430 (71)	129/310 (42)
Previous TIV vaccination, yes/total	96/432 (22)	4/310 (1)
Previous vaccination with Pandemrix, yes/total	101/387 (26)	65/271 (24)
Any past influenza vaccination recorded, yes/total	342/432 (79)	154/310 (50)

Data are presented as N (%) and n/N (%).

Abbreviations: LAIV, live attenuated influenza vaccine; TIV, trivalent inactivated influenza vaccine.

2016–2017, but increased in 2017–2018 (362 PFU/mL/day) to titers similar to the influenza B viruses (Table 2). Nearly 80% of H1-positive 2016–2017 samples contained the lowest detectable titers (133 PFU/mL/day), with only 4% of samples showing a >2-fold increase (Figure 2A). However, in 2017–2018, only 50% of H1-positive samples contained the lowest detectable titers, with 33% of samples containing at least a 4-fold increase in titer. The majority of H3-positive samples contained titers at either the lowest detectable level or 2-fold higher (Figure 2B); however, unlike H1 this was observed in both seasons (89% and 94% of samples in 2016–2017 and 2017–2018, respectively). Influenza B virus titers were similar in both seasons and demonstrated a wider distribution, with B/Vic having the highest titers (up to 6×10^4 PFU/mL/day) (Figure 2C and 2D).

Table 2. Live Attenuated Influenza Vaccine Virus Shedding and Comparison Between Influenza Seasons

Measure	Influenza Subtype	Influenza Season		aOR or Fold Difference (2017–2018 vs 2016–2017)	
		2016–2017	2017–2018	(95% CI)[b]	P Value[a]
Positivity	H1	11.2% (49/436)	3.9% (12/310)	0.34 (.16–.73)	**.005**
% of total samples (no./No.)[c]	H3	19.7% (86/436)	18.7% (58/310)	1.18 (.75–1.85)	.48
	B/Victoria	28.9% (126/436)	33.9% (105/310)	1.54 (1.05–2.26)	**.03**
	B/Yamagata	27.5% (120/436)	28.4% (88/310)	1.21 (.83–1.77)	.33
Geometric mean AUC (95% CI)	H1	204 (181–231)	362 (206–638)	1.52 (1.08–2.13)	**.02**
PFU/mL/d (95% CI)[d]	H3	263 (222–312)	224 (189–267)	0.90 (.73–1.12)	.83
	B/Victoria	719 (547–945)	466 (368–590)	1.12 (.79–1.57)	.47
	B/Yamagata	417 (340–513)	275 (230–329)	0.87 (.67–1.14)	.11

Abbreviations: aOR, adjusted odds ratio; AUC, area under the curve; CI, confidence interval; PFU, plaque-forming units.

[a]Significant results (P < .05) are in bold.

[b]Not including data from the age group 2–6 years due to low sample numbers.

[c]Adjusted odds ratio within those positive adjusted for age and any prior influenza vaccination.

[d]Fold difference in PFU/mL within those positive, adjusted for age and any prior influenza vaccination.
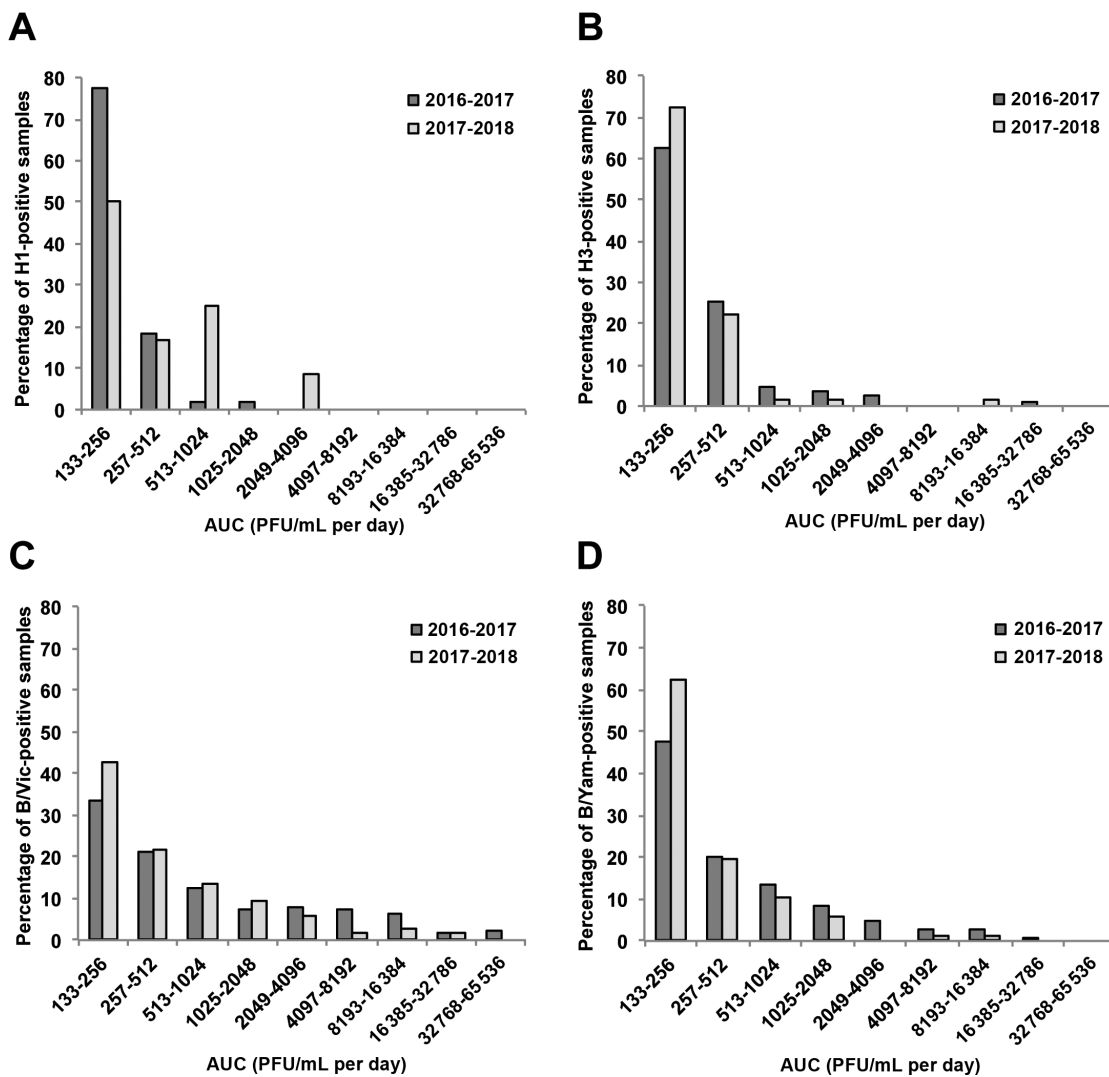

Figure 2. Analysis of viral shedding titers. The area under the curve titers (PFU/mL/day) of individual virus-positive samples were separated into 9 groups, ranging from the lowest (133 PFU/mL) to the highest observed titer for any subtype, on a 2-fold increasing scale. The percentage of samples in each group is shown as samples positive for H1 (*A*), H3 (*B*), B/Victoria (*C*), and B/Yamagata (*D*) from the 2016–2017 and 2017–2018 cohorts. Abbreviations: AUC, area under the curve; PFU, plaque-forming unit.

Adjusted odds ratio (aOR) analysis of virus positivity for 2017–2018 compared to 2016–2017 samples gave a statistically significant aOR of 0.34 for H1 (95% CI, .16–.73), showing that the H1 virus was less likely to shed in 2017–2018 than in 2016–2017 (Table 2). The aOR for H3 and B/Yam included 1.0, suggesting that these viruses were just as likely to shed in both seasons, whereas for B/Vic the aOR was >1.0, indicating higher shedding in 2017–2018 (Table 2). However, when comparing shedding titers between the 2 seasons, the AUC fold difference for H1 (1.52) was statistically significant, indicating that individuals who shed H1 were more likely to shed higher titers in 2017–2018 than in 2016–2017. The AUC fold difference for H3, B/Vic, and B/Yam ranged from 0.87 to 1.12, with none significant, suggesting little difference in the quantity of shedding between seasons.

Effects of Age and Vaccination History on Shedding

Age-stratified results (2–6, 7–11, and 12–18 years) showed that for all viruses, the proportion of virus-positive samples decreased with increasing age in 2016–2017 (Figure 3 and Supplementary Table 4). With the exception of B/Yam, the same was observed in 2017–2018. This was confirmed by logistic regression analysis where adjustment was made for any prior vaccination (Table 3).

LAIV receipt in the previous season had no effect on shedding of H1 or H3 in either season but significantly reduced shedding of both influenza B viruses in 2016–2017 and B/Vic in 2017–2018 (aOR <1) (Table 3 and Supplementary Table 5). There was no significant effect of TIV or Pandemrix receipt on any virus in either season. Results for any previous influenza vaccination were similar to those when assessing LAIV receipt in the previous season.

Serological Responses to Vaccination

In the 2016–2017 cohort, prevaccination geometric mean titers were similar between shedding-positive and -negative individuals, with the exception of B/Vic, where they were significantly lower in shedding-positive individuals (47.1 vs 88.6). Few individuals seroconverted, and although seroconversion rates were slightly higher in shedding-positive individuals, results were not significant (Table 4).

DISCUSSION

By comparing levels of the 4 viruses shed from pediatric LAIV recipients in the 2016–2017 and 2017–2018 seasons in the UK, we sought to determine if increased viral replication/shedding was observed in the clinical setting after the change in (H1N1) pdm09 (H1) strain for the 2017–2018 season. Sample positivity and virus shedding titers for H3, B/Vic, and B/Yam viruses were similar between seasons (Table 2 and Figure 2), likely due to the lack of strain changes and both cohorts containing individuals with varying vaccination histories. The 2016–2017 H1 virus, A/Bolivia/559/2013(H1N1)pdm09-like, was shed in fewer participants and at a significantly lower titer than the other 3 viruses. Although higher shedding positivity of the 2017–2018 H1 virus, A/Slovenia/2903/2015(H1N1)pdm09-like, was previously shown [19], the current study demonstrated H1 shedding from significantly fewer participants in 2017–2018 compared
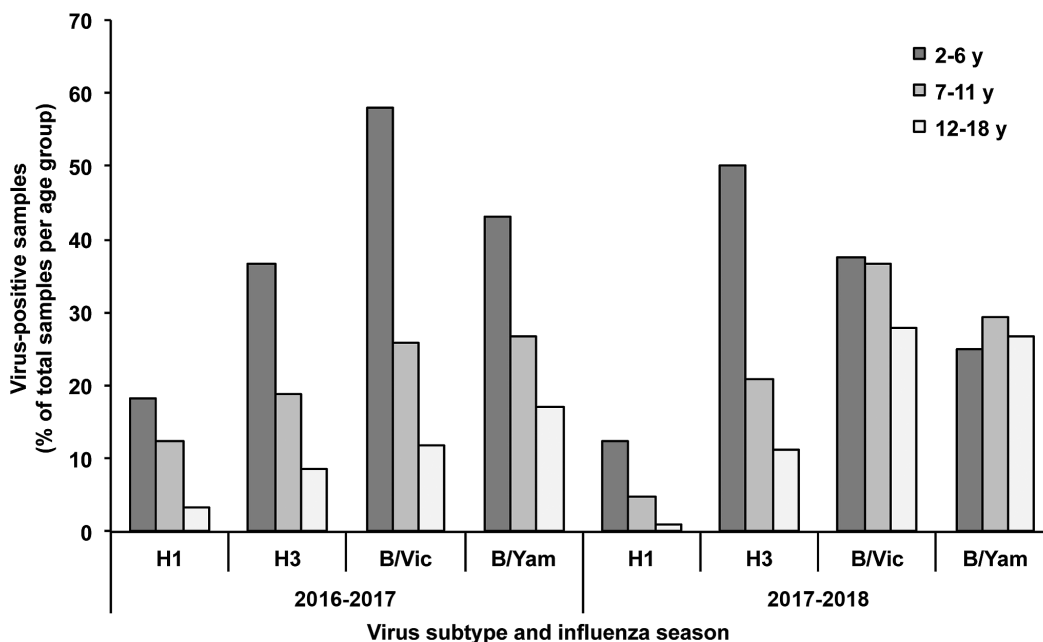

Figure 3. The effect of participant age on viral shedding. The figure shows the percentage of total samples analyzed that were positive for each subtype when stratified by age in the 2016–2017 and 2017–2018 cohorts.
Abbreviations; B/Vic, B/Victoria; B/Yam, B/Yamagata; H1 and H3, haemagglutinin subtype.

Factor	H1 aOR[a] (95% CI)[b]		H3 aOR[a] (95% CI)[b]		B/Victoria aOR[a] (95% CI)[b]		B/Yamagata aOR[a] (95% CI)[b]	
	2016–2017	2017–2018	2016–2017	2017–2018	2016–2017	2017–2018	2016–2017	2017–2018
Age, y								
2–6 vs 7–11	1.48 (.75–2.95)	2.92 (.32–26.37)	**2.13 (1.22–3.74)**	3.77 (.91–15.72)	**3.40 (2.01–5.75)**	1.10 (.25–4.81)	1.66 (.98–2.82)	0.80 (.16–4.07)
12–18 vs 7–11	**0.24 (.08–.71)**	0.18 (.02–1.48)	**0.37 (.18–.78)**	**0.48 (.23–.99)**	**0.35 (.19–.67)**	0.59 (.34–1.01)	**0.50 (.28–.89)**	0.91 (.52–1.57)
LAIV vaccination in the previous season, yes vs no	0.91 (.46–1.82)	0.77 (.23–2.54)	0.62 (.36–1.06)	1.10 (.60–1.99)	**0.50 (.30–.82)**	**0.42 (.25–.70)**	**0.51 (.31–.82)**	1.00 (.60–1.67)
Previous TIV vaccination[c], yes vs no	0.76 (.32–1.80)	…	0.64 (.32–1.28)	…	0.68 (.37–1.24)	…	0.95 (.54–1.65)	…
Previous Pandemrix vaccination, yes vs no	1.49 (.68–3.27)	0.29 (.04–2.31)	1.25 (.66–2.38)	1.04 (.51–2.13)	0.74 (.41–1.36)	0.67 (.36–1.26)	0.62 (.34–1.12)	1.68 (.91–3.08)
Any previous influenza vaccination, yes vs no	0.83 (.40–1.75)	0.58 (.18–1.91)	**0.55 (.31–.97)**	0.97 (.54–1.75)	**0.52 (.30–.90)**	**0.51 (.31–.83)**	**0.43 (.26–.72)**	1.14 (.69–1.89)

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; LAIV, live attenuated influenza vaccine; TIV, trivalent inactivated influenza vaccine.

[a]Adjusted for age when assessing previous vaccination and for any previous influenza vaccine when assessing age.

[b]Significant results ($P < .05$) are in bold.

[c]Lack of TIV aOR data for 2017–2018 due to the low number of prior TIV recipients in this cohort.

with 2016–2017 (Table 2). However the study design differed between these 2 studies, which must be taken into consideration when comparing results. The previous study [19] was designed to minimize the impact from prior infection or vaccination, whereas the current study aimed to assess shedding in both vaccine-naive and twice LAIV-vaccinated individuals to address the effect of prior vaccination on LAIV virus shedding. Furthermore, the previous study was performed in the summer months, whereas the current study was performed during the influenza season and reflected the real-world use of LAIV in the UK. As a result, the cohorts in the 2 studies differed in participant age, vaccination history, and serostatus, all of which are likely to impact virus shedding. This illustrates the complexity of studying vaccine responses in different cohorts, as even similar cohorts vaccinated with the same vaccine but in different countries have demonstrated significantly different VE results [16, 25]. Nevertheless, results of the current study demonstrated that in those individuals who did shed H1, the average titer was higher than the previous year, consistent with the findings that the 2017–2018 strain change increased H1 replication [19]. However, the significantly lower shedding positivity in 2017–2018 warrants further investigation regarding the implications for VE.

A significant negative association between participant age and shedding was observed (Table 3). Younger individuals are less likely to have undergone repeated influenza exposure through vaccination or infection, and therefore less likely to have preexisting immunity. This would be consistent with higher LAIV VE observed in children compared to 18- to 49-year-olds [26]. However, the negative association between shedding and age was independent of vaccination history (Table 3) and for 3 of the viruses, no association between prevaccination antibody titers and shedding was observed (Table 4). The factors responsible for the age effect are therefore unclear and require further investigation.

Prior vaccination was associated with reduced shedding, at least for influenza B viruses, independently of age. This was not the case for (H1N1)pdm09. If the effect of prior LAIV on shedding is mediated by preexisting immunity, it may depend on cell-mediated or mucosal antibody responses, factors not measured in our study, as there was no relationship between prevaccination titers and shedding. Furthermore, prior receipt of TIV or Pandemrix, a monovalent ASO3-adjuvanted vaccine associated with elevated neutralizing antibody levels to (H1N1) pdm09 [23], also had no effect on shedding. Preexisting cellular immunity has been shown to negatively correlate with LAIV-induced T-cell responses in children but not adults [27].

The minimal seroconversion observed, regardless of shedding status, demonstrates that vaccine virus shedding levels and LAIV-mediated serum antibody responses do not correlate with the high (H1N1)pdm09 VE observed in the UK during the 2017–2018 season (Supplementary Table 1) [22]. Serum antibody titers and

Table 4. Serological Analysis of Live Attenuated Influenza–Vaccinated Participants

Measure and Assay	HA Subtype	Strain	Shedding Negative Samples	Shedding Positive Samples	P Value[a]
Geometric mean prevaccination antibody titer (95% CI)					
HAI	H1	A/California/07/2009 (H1N1)pdm09	65.3 (48.4–88.1)	72.9 (40–132.9)	.79
	H1	A/Bolivia/559/2013	50.7 (38.8–66.4)	50.4 (28.9–87.8)	.91
	H3	A/Hong Kong/4801/2014	187.1 (154.8–226)	149.3 (93.4–238.6)	.31
	B/Victoria	B/Brisbane/60/2008	88.6 (71.1–110.3)	47.1 (28.6–77.5)	**.009**
	B/Yamagata	B/Phuket/3073/2013	54.6 (43.2–69)	45.1 (31.1–65.4)	.42
NAI	H1	A/Bolivia/559/2013	152.7 (105.1–222)	312.7 (121.6–804)	.19
Seroconversion, no./No. (%)					
HAI	H1	A/California/07/2009 (H1N1)pdm09	2/96 (2)	1/15 (7)	.36
	H1	A/Bolivia/559/2013	5/94 (5)	1/15 (7)	1.00
	H3	A/Hong Kong/4801/2014	2/91 (2)	0/23 (0)	1.00
	B/Victoria	B/Brisbane/60/2008	13/85 (15)	6/28 (21)	.56
	B/Yamagata	B/Phuket/3073/2013	9/86 (10)	7/28 (25)	.07
NAI	H1	A/Bolivia/559/2013	4/98 (4)	0/15 (0)	1.00

Abbreviations: CI, confidence interval; HAI, hemagglutination inhibition; NAI, neuraminidase inhibition.
[a]Significant results ($P < .05$) are in bold.

seroconversion rates are known to be poor correlates of protection in LAIV-vaccinated children [17], and a lack of correlation between mucosal antibody, cytokine or chemokine levels, and protection has previously been noted [28]. Results of this study suggest that attempting to correlate quantitative virus shedding with clinical protection may be a complex process requiring consideration of multiple factors, such as recipient age and vaccination history. In future studies, if virus shedding is to be investigated as an indicator of LAIV viral strain replicative fitness and a potential correlate of protection, the focus should be on LAIV-naive children aged 2–6 years, in whom shedding rates are highest.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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