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Responses to live attenuated influenza vaccine in children vaccinated previously with Pandemrix ($ASO3_B$ adjuvanted pandemic A/H1N1pdm09)

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ABSTRACT

Background: We report a phase III/IV open-label study on the immunogenicity of a single dose of a Live Attenuated Influenza Vaccine (LAIV) (Fluenz™) in children naïve to, or in previous receipt of, $AS03_B$ adjuvanted A/H1N1pdm09 influenza vaccine (Pandemrix™), to investigate whether early exposure to an adjuvanted subunit influenza vaccine impacts on subsequent response to quadrivalent LAIV (qLAIV).
Method and findings: Eligible children were enrolled to receive qLAIV and stratified according to previous Pandemrix™ vaccination. Functional antibody for the vaccine strains were analysed using Haemagglutination Inhibition (HAI); in addition antibodies to the A/H1N1pdm09 strain were measured by Neuraminidase Antibody Inhibition (NAI) and neutralisation assays. Fourfold titre increases by HAI were observed for 39% (95% confidence interval 33–46%) and 43% (37–51%) of subjects for the two influenza B vaccine strains and 8% (5–13%) for the A/H3N2 strain with no significant differences between the Pandemrix™ naïve or previously vaccinated groups in antibody tites pre- or post-vaccination or seroconversion rates. In both groups, the response to the qLAIV A/H1N1pdm09 component was barely detectable, overall HAI seroconversion rate 1.8% (0.5–4.7%). Previous receipt of Pandemrix™ was associated with significantly higher levels of A/H1N1pdm09 neutralising antibody, but decreased NAI titres pre-vaccination, with the differences maintained post-vaccination.
Conclusion: Previous receipt of Pandemrix™ has had a significant impact on the influenza immune status of children several years later. Higher levels of neutralising antibody to A/H1N1pdm09 pre- and post-vaccination, but significantly lower levels of antibody to NA, were observed compared with Pandemrix™-naïve children, while responses to influenza B and A/H3N2 and antibody levels prior to vaccination were similar in both groups. This suggests that early vaccination with a powerful adjuvant maintains functional immunity for several years, which prevents natural infection. Alternatively, the $AS03_B$ adjuvant may have re-directed the immune response, with focus towards viral HA and away from viral NA.

1. Introduction

In June 2012, the United Kingdom's Committee for Vaccination and Immunization (JCVI) recommended the annual vaccination of all children aged 2–16 years using the live attenuated influenza vaccine (LAIV) [1]. LAIV was chosen for ease of administration and evidence of superior efficacy compared with the inactivated influenza vaccine (IIV) [2]. The programme was implemented as a phased roll-out in pilot areas beginning in the 2013/14 season in children aged 2–3 years. Extension to older birth cohorts has taken place in successive years [3]. A single dose of the quadrivalent LAIV (qLAIV) containing an A/H1N1pdm09, A/H3N2 and two B strains has been offered to eligible age groups irrespective of prior vaccination history.

Since little is known about the immunological response to repeated annual LAIV and how this relates to protection from natural infection, a cohort of children was established in 2014/15 to measure antibody responses to successive LAIV vaccinations. We also wished to investigate whether previous vaccination of young

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children with monovalent pandemic vaccine in 2009/10 ($AS03_B$ adjuvanted A/H1N1pdm09 pandemic vaccine; Pandemrix™) impacted on responses to qLAIV given several years later. Our previous work unexpectedly demonstrated that receipt of Pandemrix™ in 2009/10 enhanced responses to heterologous A/H3N2 and B components as well as homologous (A/H1N1pdm09) components of trivalent inactivated influenza vaccine (TIV), given a year later in the youngest children [4], likely as a result of the powerful adjuvant activity associated with $ASO3_B$. In contrast to IIV, antibody responses to Pandemrix™ were highest in the youngest infants [5] indicating that such vaccines have a profound effect on the naive immune system.

In this paper we study the impact of prior Pandemrix™ on the serological responses to homologous and heterologous vaccine antigens in children aged between 5 and 10 receiving qLAIV.

2. Materials and methods

2.1. Trial approvals

The study was sponsored by Public Health England (PHE) and approved by the UK Medicines and Healthcare Products Regulatory Agency (EudraCT number 2013-003592-35), the NRES Committee London - West London & GTAC (14/LO/0227) and was given local NHS approval.

2.2. Study design

Study participants were recruited and vaccinated by research nurses based in primary care in Gloucestershire, Hertfordshire and North West London. The inclusion criteria were aged 5 to <11 years on the day of recruitment, with written informed consent given by parent/legal guardian and either documented prior receipt of Pandemrix™ or no evidence in the medical notes of ever having had any monovalent pandemic influenza vaccine. Exclusion criteria were hypersensitivity to any of the vaccine constituents, clinical immuno-deficiencies, recipients of salicylate therapy, or any other vaccine within a month prior to LAIV or any contraindication to vaccination as specified in the "Green Book"- Immunisation against Infectious Disease [6]. Each participant received one dose of qLAIV (during the period of October 2014 to February 2015). Blood samples were collected on the day of immunization and on day 21 after vaccination.

2.3. Vaccine

Participants were given quadrivalent live attenuated influenza vaccine (Fluenz Tetra®, AstraZeneca UK Limited, batch JH2616) containing an A/California/7/2009(H1N1)pdm09-like virus ($H1N1_{Cal}$), an A/Texas/50/2012(H3N2)-like virus ($H3N2_{Texas}$) and a B/Massachusetts/2/2012-like B/Yamagata-lineage virus (B_{Mass}), plus a B/Brisbane/60/2008-like B/Victoria-lineage virus (B_{Bris}) intra-nasally as a 0.1 mL spray dose into each nostril.

2.4. Laboratory analysis

Serological analysis was performed at the National Infection Service of Public Health England (London, UK). Antigens for all assays were egg-grown; influenza B antigens were Tween80/Diethyl-Ether extracted prior to their use in Haemagglutination inhibition (HAI) assays by adding Tween® 80 to final concentrations of 0.125%, incubation for 20 min at room temperature and addition of a half volume of Diethyl ether (ACS reagent). The mixtures were stirred for a minimum of 45 min inside a fume cupboard and then left standing until the phases had fully separated and the aqueous phase could be extracted for use as antigen. HAI assays were performed using previously described methods [4]. Briefly, sera treated to remove non-specific inhibitors were twofold serially diluted starting at a 1:10 dilution then mixed with an equal volume (25 μl) of PBS containing 4HA units of each of the vaccine strains and A/Switzerland/9715293/2013(H3N2) virus ($H3N2_{Switz}$), a strain that started to emerge in the UK during the 2014/15 season [7]. Turkey red blood cells (RBC) were used for the influenza A/H1N1pdm09 and influenza B components, and Guinea pig RBC for H3N2. HAI titres were expressed as the reciprocal of the last serum dilution that gave complete inhibition of agglutination.

Microneutralisation (MN) and Neuraminidase Antibody Inhibition (NAI) assays were performed for the A/H1N1pdm09 vaccine component only. The MN assay was performed using previously described methods [8] and titres were reported as reciprocal dilution at which 50% of virus was neutralised.

For the NAI analysis, an enzyme-linked lectin assay (ELLA) [9] was performed using a reverse genetics virus – NIBRG127 (H7N1), gift from NIBSC, Potters Bar, UK expressing neuraminidase [NA] from A/California/7/2009. Briefly, 96-well plates were coated with fetuin, and a standard amount of egg grown virus mixed with heat treated sera (56 °C, 30 min) which had been serially diluted (twofold; starting at 1:10). Plates were then incubated at 37 °C overnight (16–20 h). Remaining viral NA activity was detected by binding of horseradish peroxidase-conjugated peanut agglutinin lectin (PNA-HRPO), followed by addition of 3,3′,5,5′-Tetramethyl benzidine Liquid Substrate (TMB) and analysis in a spectrophotometer at an optical density (OD) of 450 nm. Every serum was analysed in independent duplicate assays and the reciprocal of the highest serum dilution that resulted in $\geq$ 50% inhibition of viral NA activity, IC50 (calculated using linear regression in Microsoft Excel 2010) was designated as the NAI antibody titre. HAI, MN and NAI titres < 10 were assigned a value of 5.

2.5. Statistical analysis

The percentage of subjects with a fourfold rise in antibody titres from pre- to post-vaccination was calculated and compared by prior Pandemrix™ status using Fisher's exact test. Geometric mean titres pre- and post-vaccination as well as geometric mean fold change pre- to post-vaccination were calculated with 95% confidence intervals, and compared between those who received and did not receive Pandemrix™ using the Kruskall-Wallis test. The correlation between HAI, MN and NAI titres was assessed in pre- and post-vaccination sera according to Pandemrix™ vaccination status using Pearson's correlation coefficient. Normal errors on the logarithm of the pre- and post-vaccination titres was undertaken to look at the relationship between this and month of sample (Oct/Nov and Dec/JanFeb), prior Pandemix™ (overall and by whether given at age < 4 or $\geq$ 4 years), other influenza vaccination (Inactivated Influenza vaccine; IIV) in previous years (yes/no), and age in years at LAIV vaccination.

3. Results

3.1. Participants

A total of 256 participants aged between 5 and 10 years were recruited to the study from October 2014 to February 2015, two of whom subsequently withdrew prior to vaccination. Of the remaining 254 participants, 240 provided a pre-vaccination blood sample and 227 a post-vaccination sample for analysis (Fig. 1); 217 subjects provided evaluable serum pairs. Characteristics of the two groups stratified by prior Pandemrix™ receipt (yes/no), were broadly similar and are shown in Table 1. The number of

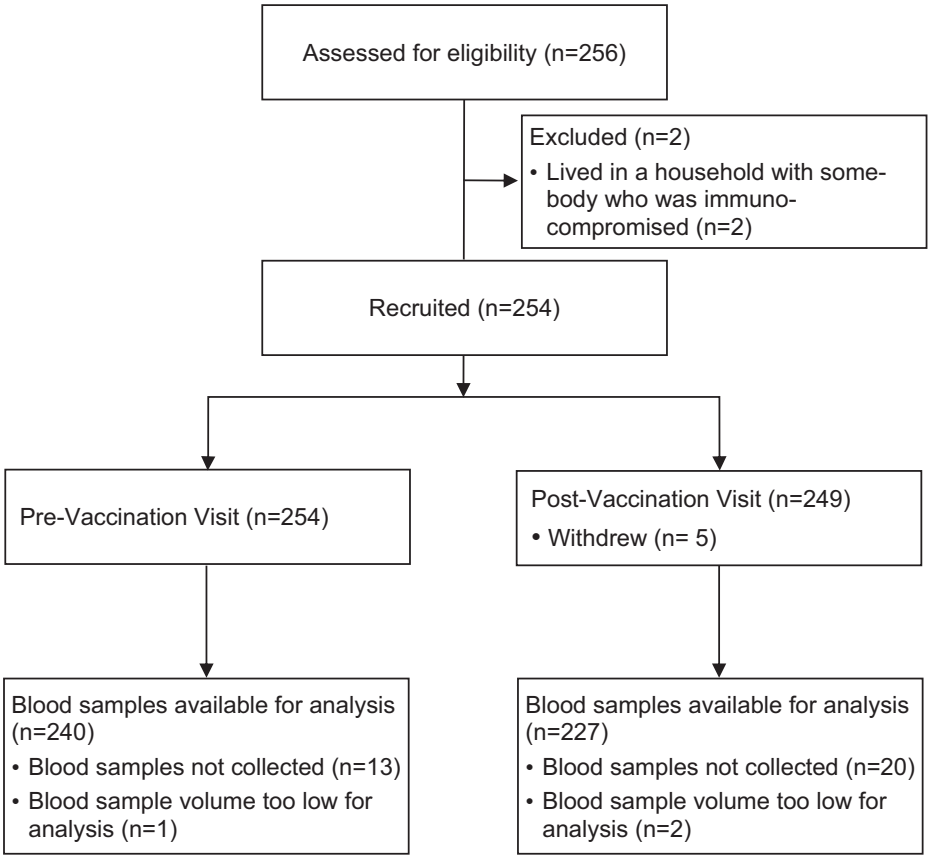

Fig. 1. Flow of participants. Children were recruited to two groups, which were defined by their vaccination history – vaccinated with Pandemrix (n = 97), or naïve to pandemic influenza vaccine (n = 157). (All participants received the same treatment; (EudraCT number 2013–003592-35)).

Table 1
Characteristics of the 254 participants who received qLAIV and follow up procedures according to Pandemrix™ vaccination status.

Characteristics	Prior Pandemrix™ N = 97	Pandemrix™ naive N = 157
Male:female (percentage male)	51:46 (53%)	73:84 (46%)
Age Median (range)	7 years (5–10)	8 years (5–10)
Age at qLAIV No. (%)		
5 - <7 years	35 (36%)	48 (31%)
7 - <9 years	38 (39%)	45 (29%)
9 - <11 years	24 (25%)	64 (41%)
LAIV given:		
Oct - Nov	9 (9%)	18 (11%)
Dec - Feb	88 (91%)	139 (89%)
Evaluable blood sample		
Pre LAIV No. (%)	95 (98%)	145 (92%)
Post LAIV No. (%)	92 (95%)	135 (86%)
Interval qLAIV to blood sample		
Median (range)	22 days (20–49)	23 days (18–57)
Prior inactivated influenza vaccine	6 (6%)	11 (7%)

children who had received IIV previously was 11% in the Pandemrix™ naïve group, compared with 6% in the children who had received Pandemrix™.

3.2. Serological responses measured by HAI, MN and NAI

Before vaccination, HAI antibody levels were generally high in all children, with between 51% and 90% participants having titres ≥40 to each of the four vaccine strains (Table 2). Pre- vaccination antibody titres to A/H1N1pdm09 and A/H3N2 were markedly higher than those to influenza B, whereas rises in geometric mean titres (GMT) and seroconversion rates for HAI antibodies were more pronounced for the influenza B than A strains where only modest levels of seroconversion were seen for both A/H3N2 strains (8.3 and 9.2% respectively), and only 1.8% for influenza A/H1N1pdm09. Seroconversion rates by HAI to the influenza A viruses were significantly higher in those with pre-vaccination titres <40 than in those with titres ≥40: 7% (3/43) versus 0.6% (1/174) for $H1N1_{Cal}$ ($p = 0.03$) and 38% (8/21) versus 5% (10/196) for $H3N2_{Texas}$ ($p < 0.01$). The seroconversion rate measured by MN for A/H1N1pdm09 was also low (0.9%), but higher (7.1%) when measured by $H1N1_{Cal}$ NAI.

3.3. Comparison of antibody responses by Pandemrix™ vaccination status

Analysis of results stratified according to prior Pandemrix™ exposure (Table 3 and Fig. 2) shows that there were differences in $H1N1_{Cal}$ antibody levels between the two groups before and after qLAIV vaccination. $H1N1_{Cal}$ titres as measured by MN and HAI pre- and post-vaccination were highly correlated in both groups (R-squared ≥0.91 for all four correlations) while the correlation between NAI and HAI was much lower (R-squared <0.47 for Pandemrix™ naïve and <0.36 for Pandemrix™ exposed). The pre-vaccine HAI and MN antibody GMTs to $H1N1_{Cal}$ were higher in the Pandemrix™ exposed than the naïve group though this only reached statistical significance for MN. In contrast, the pre-vaccination NAI antibody GMT was significantly lower in the Pandemrix™ exposed than naïve group. These differences persisted post–vaccination. There were no significant differences between the prior Pandemrix™ and naïve groups in vaccine response as measured by fold change in titre (Table 3).

Table 2
Pre vaccination and post vaccination proportions with titres ≥ 40 and geometric mean titres (GMTs) with 95% confidence intervals (CIs) as measured by HAI, MN and NAI together with geometric mean fold changes pre to post vaccination and proportions seroconverting (≥4-fold rise in titre).

Test: strain	Pre-vaccination ≥ 40% (95% CI)	Pre-vaccination GMT (95% CI)	Post vaccination ≥ 40% (95% CI)	Post-vaccination GMT (95% CI)	Geometric Mean Fold change in GMTs[a](95% CI)	Seroconversion rate (95% CI)
HAI: $H1N1_{Cal}$	190/240 79.2% (73.5–84.1%)	84.9 (70.5–102.4)	186/227 81.9% (76.3–86.2%)	91.6 (76.3–109.9)	1.10 (1.04–1.18)	4/217 1.8% (0.5–4.7%)
MN: $H1N1_{Cal}$	212/239 88.7% (84.0–92.4%)	264.9 (214.5–327.2)	204/225 90.7% (86.1–94.1%)	291.4 (237.0–358.3)	1.14 (1.07–1.21)	2/215 0.9% (0.1–3.3%)
NAI: $H1N1_{Cal}$	184/238 77.3% (71.5–82.5%)	251.1 (186.0–338.8)	168/223 75.3% (69.1–80.9%)	269.5 (194.4–373.4)	1.19 (1.07–1.32)	15/212 7.1% (4.0–11.4%)
HAI: $H3N2_{Texas}$	215/240 89.6% (85.0–93.1%)	170.3 (141.2–205.4)	214/227 94.3% (90.4–96.9%)	227.1 (192.3–268.1)	1.27 (1.12–1.44)	18/217 8.3% (5.0–12.8%)
HAI: $H3N2_{Switz}$	147/240 61.3% (54.8–67.5%)	45.0 (36.7–55.3)	157/227 69.2% (62.7–75.1%)	60.2 (48.9–74.1)	1.27 (1.13–1.44)	20/217 9.2% (5.7–13.9%)
HAI: B_{Bris}	119/233 51.1% (44.5–57.7%)	31.2 (25.1–38.9)	163/220 74.1% (67.8–79.8%)	94.9 (76.4–118.0)	3.02 (2.56–3.56)	81/206 39.3% (32.6–46.4%)
HAI: B_{Mass}	134/232 57.8% (51.1–64.2%)	40.8 (32.8–50.8)	194/219 88.6% (83.6–92.5%)	151.3 (125.8–182.0)	3.82 (3.13–4.67)	89/205 43.4% (36.5–50.5%)

[a] For those with paired samples only, numbers as in seroconversion rate column.

Table 3
Geometric mean titres and fold change pre and post vaccination (with 95% confidence intervals) according to history of prior Pandemrix™ vaccination.

Strain	Prior Pandemrix™	Pre-vaccination GMT Prior Pandemrix™ N = 95 No Pandemrix™ N = 145	P-value*	Post-vaccination GMT Prior Pandemrix™ N = 92 No Pandemrix™ N = 135	P-value*	Fold change (GM change) Prior Pandemrix™ N = 90[a] No Pandemrix ™ N = 127	P-value*
HAI: $H1N1_{Cal}$	Yes No	104.9 (78.1–141.0) 73.9 (58.1–94.1)	0.15	102.2 (76.6–136.4) 85.0 (67.0–107.8)	0.57	1.09 (0.98–1.23) 1.11 (1.03–1.20)	0.32
MN: $H1N1_{Cal}$	Yes No	**422.2 (317.6–561.4)** **194.8 (146.1–259.7)**	**0.007**	**412.6 (309.8–549.3)** **230.1 (173.4–305.5)**	**0.05**	1.13 (1.00–1.28) 1.15 (1.08–1.22)	0.23
NAI: $H1N1_{Cal}$	Yes No	**88.2 (53.3–146.2)** **317.8 (231.8–535.7)**	**0.0001**	**83.7 (49.4–141.6)** **388.0 (275.0–547.5)**	**0.0001**	1.15 (0.98–1.34) 1.22 (1.07–1.38)	0.08
HAI: $H3N2_{Texas}$	Yes No	181.6 (135.5–243.5) 163.3 (127.6–208.8)	0.72	240.9 (188.1–308.5) 218.2 (174.2–273.3)	0.54	1.26 (1.05–1.50) 1.27 (1.07–1.51)	0.67
HAI: $H3N2_{Switz}$	Yes No	50.1 (36.3–69.3) 42.0 (32.1–54.9)	0.49	66.7 (48.5–91.8) 56.1 (42.5–74.0)	0.33	1.23 (1.06–1.42) 1.31 (1.09–1.57)	0.87
HAI: B_{Bris}	Yes No	32.1 (22.9–45.0) 30.7 (22.9–41.1)	0.76	91.7 (66.5–126.5) 97.1 (72.3–130.5)	0.51	2.85 (2.15–3.77) 3.14 (2.57–3.85)	0.34
HAI: B_{Mass}	Yes No	44.7 (31.6–63.2) 38.4 (28.9–51.2)	0.59	154.7 (115.5–207.1) 149.1 (117.2–189.8)	0.88	3.88 (2.74–5.50) 3.78 (2.97–4.81)	0.97

Significant differences between groups (prior/no Pandemrix™) are indicated in bold.
* P-values compare titres or fold changes by prior Pandemrix™ status using the Kruskal-Wallis test.
[a] While Pre-and Post-vaccination titres are calculated for all available samples, fold changes are for those with paired samples only.

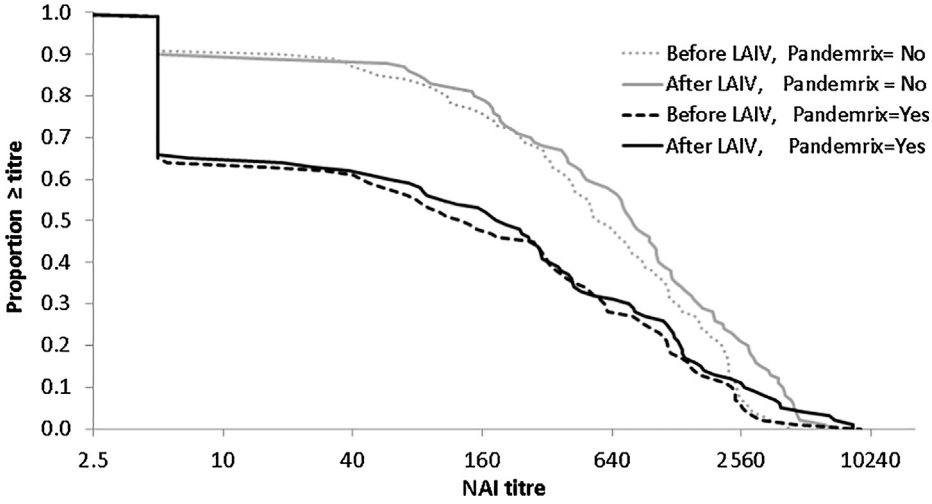

Fig. 2. Reverse cumulative distribution curves pre and post qLAIV according to Pandemrix™ vaccination status for the A/$H1N1_{Cal}$ titres as measured by neuraminidase inhibition (NAI) assay.

Table 4
Adjusted[a] geometric mean fold differences (95% CI) pre and post vaccination according to prior seasonal influenza vaccination and prior Pandemrix™ vaccination.

Timing of blood sample	Prior vaccination status	HAI: $H1N1_{Cal}$	NAI: $H1N1_{Cal}$	HAI: $H3N2_{Texas}$	HAI: $H3N2_{Switz}$	HAI: B_{Bris}	HAI: B_{Mass}
Pre vaccination							
Prior seasonal influenza vaccination	No (N = 223)	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
	Yes (N = 17)	1.42 (0.69–2.93)	1.52 (0.53–4.34)	2.34 (1.14–4.83)	2.13 (0.98–4.66)	0.97(0.43–2.21)	2.75 (1.17–6.49)
Prior Pandemrix™ vaccination	No (N = 145)	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
	Yes: aged <4 (N = 63)	1.20 (0.78–1.85)	0.19 (0.10–0.35)	1.07(0.69–1.66)	1.42 (0.88–2.28)	0.77(0.46–1.28)	1.15 (0.69–1.91)
	Yes: aged ≥ 4 (N = 32)	1.89 (1.08–3.32)	0.56 (0.25–1.27)	1.17(0.67–2.07)	0.83(0.45–1.53)	1.84(0.95–3.58)	1.11(0.57–2.14)
	Yes: any age (N = 95)	1.41(0.97–2.07)	0.31 (0.18–0.54)	1.14(0.78–1.67)	1.10(0.73–1.66)	1.15 (0.74–1.78)	1.14 (0.73–1.78)
Post vaccination							
Prior seasonal influenza vaccination	No (N = 212)	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
	Yes (N = 15)	2.10 (1.00–4.41)	1.84 (0.59–5.73)	1.84 (0.94–3.60)	1.84 (0.80–4.20)	2.56 (1.10–5.97)	2.15 (0.99–4.66)
Prior Pandemrix™ Vaccination	No (N = 135)	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
	Yes: aged <4 (N = 61)	1.01(0.66–1.55)	0.14 (0.07–0.29)	1.04 (0.70–1.53)	1.40(0.86–2.28)	0.72 (0.43–1.19)	1.05 (0.68–1.62)
	Yes: aged ≥4 (N = 31)	1.95(1.13–3.36)	0.50 (0.21–1.21)	1.02 (0.62–1.68)	0.73(0.39–1.36)	1.47(0.75–2.86)	1.07 (0.60–1.91)
	Yes any age (N = 92)	1.33(0.99–1.94)	0.29 (0.16–0.52)	1.02 (0.72–1.44)	0.99 (0.64–1.51)	1.06 (0.68–1.66)	1.04 (0.70–1.54)

[a] Adjusted for month of sample, age at LAIV vaccination.

For influenza B and A/H3N2 antibody responses, both groups had similar HAI GMT antibody titres prior to LAIV vaccination, and made similar rises in post-vaccine responses, with influenza B responses being more evident than H3N2 responses (Table 3, Fig. 2b).

The lower pre-vaccination titres to $H1N1_{Cal}$ as measured by NAI in the Pandemrix™ exposed group were confirmed in the linear regression model (Table 4). In children who received Pandemrix™ at age ≥4 years, pre-vaccination HAI titres to $H1N1_{Cal}$ were significantly higher than in Pandemrix™ naïve children. These pre-vaccination differences in NAI and HAI persisted post-vaccination. NAI pre-vaccination titres in both the prior Pandemrix™ and Pandemrix™ naïve groups increased with age (p = 0.003 for each respectively), but not as measured by HAI (p = 0.12 and 0.82 respectively). Prior seasonal vaccination with IIV was also generally associated with higher pre-qLAIV titres, although only significantly so for $H3N2_{Texas}$ and B_{Mass}. Timing of the pre-qLAIV sample only showed a significant effect for $H3N2_{Switz}$ GMT with a fold effect of 2.17 (95% CI 1.15–4.11, p = 0.018) for the period December 2014 to February 2015 compared with October/November as the reference.

4. Discussion

LAIV is believed to induce protective responses dependent on local replication of the virus. Consequently, clinical studies have focused on the quantification of local immune responses (such as mucosal antibody) and also markers of cell mediated responses, but correlates of protection have not yet been defined [10]. In our study we assessed "classic" serological markers such as HAI, MN and NAI antibody in vaccinated children, stratified by their previous exposure to an adjuvanted subunit vaccine, Pandemrix™, at an early age during the 2009/10 winter season, when the use of this vaccine was part of the UK response to the pandemic of 2009. Receipt of Pandemrix™ had a long lasting immunological impact as shown by the significantly lower NAI levels pre- and post-qLAIV vaccination and significantly higher MN titres in Pandemrix™ vaccinated than naïve children (Table 3). In both groups seroconversion rates to $H1N1_{Cal}$ were low and paralleled the observed LAIV vaccine effectiveness in the field with B > A/H3N2 > A/H1N1pdm09 [7,11].

Pandemrix™ was widely used in the UK pandemic vaccination program with about a quarter of healthy children aged <5 years vaccinated in 2009/10 in England [12]. Previously, we reported that exposure to monovalent adjuvanted pandemic vaccine enhanced subsequent responses to unrelated influenza vaccine components in children given IIV a year later [4]. In the current study, conducted some five years after Pandemrix™ receipt, we did not find significant enhancement of HAI antibody responses to either homologous or heterologous HA antigens administered via qLAIV, but did see effects on $H1N1_{Cal}$ specific NAI and MN antibodies. Neutralisation assesses a wider repertoire of anti-HA antibodies than those measured by HAI, which largely measures antibodies to the receptor binding region of the head of the viral HA. Both of these antibody measures were highly correlated, unlike HAI and NAI, suggesting independent induction mechanisms for NAI in our study children. In adults, antibodies to HA and NA are induced by natural infection and both are independently involved in protection [13,14]. We have previously shown that Pandemrix™ vaccine resulted in increases in both these antibodies in adults in the intervals immediately following vaccination [15], but similar data does not exist for children.

Vaccination with Pandemrix™ induced high levels of A/H1N1pdm09 antibody [5], and high vaccine effectiveness which persisted over the next H1N1 pandemic influenza season [16]. One explanation for the high neutralising antibody, but low NA

titre in Pandemrix™ immunised children, is that the durable protection provided by the high HA vaccine-antibody levels limited the stimulus for production of antibodies to viral NA through natural infection so that NA antibody levels were not boosted. In contrast, Pandemrix™ naïve children would not have high levels of protective HA antibody, other than those acquired through natural infection, and would be more susceptible to influenza A/H1N1pdm09 strains which have dominated the winter influenza seasons in several of the years following 2009 with viral replication boosting NA antibodies. The impact of natural infection with circulating strains was demonstrated by the twofold increase in HAI antibodies to $A/H3N2_{Switz}$ in pre-vaccination sera taken after its appearance in the UK. We also found that pre-vaccination NAI antibody levels increased with age in both Pandemrix™-vaccinated and Pandemrix™-naïve children consistent with acquisition though natural exposure. Although an increase with age has been reported previously for NAI antibodies it has not been described for the narrow age bracket (5 to <11 years of age) in our study [17,18]. It is also possible that in the presence of a powerful adjuvant coupled with a subunit HA protein, the initial antibody repertoire towards viral HA may be broader and generate higher potency protective antibodies, which may in turn divert the B memory cell antibody response away from competing viral antigens [19] such as the viral NA in subsequent infections.

We found evidence of antibody persistence after previous seasonal vaccination using linear regression models, with previous receipt of vaccine resulting on average in a more than doubling of pre-qLAIV titres with B_{Bris} and $H3N2_{Texas}$ strain and similar effect for post-qLAIV HAI antibody levels with B_{Bris}, B_{Mass} and $H1N1_{Cal}$ strains, all of which would have been vaccine components in previous years.

Our serology data from vaccinees is in line with recent assessments of VE: we detected significant increases in serological correlates of protection with influenza B_{Bris}, B_{Mass} and, to a lesser degree with $H3N2_{Texas}$, but not with $H1N1_{Cal}$. Poor responses to the A/H1N1pdm09 component of qLAIV has been shown for pre-pandemic H1N1 strains yet these vaccines have provided reasonable protection as measured by VE in the field against H1N1 influenza [20], exemplifying the conundrum of describing a correlate of protection for LAIV.

It has been suggested that the A/H1N1pdm09 qLAIV vaccine strains have poor replicative fitness compared with pre-pandemic H1N1 strains and that replacement with an A/H1N1pdm09 strain with better replicative fitness would improve protection, as one possible hypothesis for the poor performance of qLAIV in the USA [21]. An alternative, though not mutually exclusive hypothesis, is that poor VE may be consequent on prior vaccination history of recipients, and high levels of immunity may prevent or limit qLAIV viral replication, thus limiting the induction of functional immunity to circulating strains. This is supported by our finding that seroconversion rates to influenza A viruses were significantly lower in children with pre-vaccination HAI antibody above the serological threshold of protection used for IIV (titre ≥ 40).

Measurement of the strain specific VE of qLAIV in the field, in parallel with comparative assessment of vaccine virus shedding in recipients and the serological, mucosa and cell-mediated responses to the vaccine strains will provide insights into the relationship between viral replication in the upper airways and induction of protective immunity.

5. Conclusion

The unique design of our study revealed that prior receipt of the $AS03_B$-adjuvanted A/H1N1pdm09 vaccine influences antibody levels five years later and results in low baseline levels of NAI antibody and high levels of neutralising antibody. This suggests protection from infection with A/H1N1pdm09 is durable over several subsequent seasons for those children previously vaccinated with Pandemrix™, while Pandemrix™-naïve children acquired antibody against viral NA, from natural infection. Redirection of the immune response as a long lasting effect of adjuvant resulting in the focus of subsequent responses towards the HA may also be relevant. The long term consequences of these differences in early priming of the immune system *in vivo* are unclear as antibodies to neuraminidase contribute to protection.

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Author Contributions

JS drafted the protocol, obtained all governance approvals and over saw the field work and data entry. KH was responsible for the laboratory testing, involved in data interpretation and produced the first draft of the paper. CT optimised some of the methods used in the laboratory analyses for NA antibody. FW and NA conducted the statistical analysis. EM, MZ designed the study and with NA directed the analyses and interpretation of the results. All authors contributed to, reviewed and approved the final draft of the manuscript.

Competing Interest

NA, FW, JS, EM, KH, CT have no conflicts of interest. Between 2000 and 2008 MZ has been investigator of clinical trials sponsored by Novartis, Baxter, Sanofi Pasteur and CSL Australia Ltd. All grants and honoraria are paid into accounts of their employer (HPA/PHE) and none received personally.

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