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Association of vaccine handling conditions with effectiveness of live attenuated influenza vaccine against H1N1pdm09 viruses in the United States

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ABSTRACT

Purpose: This analysis examined potential causes of the lack of vaccine effectiveness (VE) of live attenuated influenza vaccine (LAIV) against A/H1N1pdm09 viruses in the United States (US) during the 2013–2014 season. Laboratory studies have demonstrated reduced thermal stability of A/California/07/2009, the A/H1N1pdm09 strain utilized in LAIV from 2009 through 2013–2014.

Methods: Post hoc analyses of a 2013–2014 test-negative case-control (TNCC) effectiveness study investigated associations between vaccine shipping conditions and LAIV lot effectiveness. Investigational sites provided the LAIV lot numbers administered to each LAIV recipient enrolled in the study, and the vaccine distributor used by the site for commercially purchased vaccine. Additionally, a review was conducted of 2009–2014 pediatric observational TNCC effectiveness studies of LAIV, summarizing effectiveness by type/subtype, season, and geographic location.

Results: From the 2013 to 2014 TNCC study, the proportion of LAIV recipients who tested positive for H1N1pdm09 was significantly higher among children who received a lot released between August 1 and September 15, 2013, compared with a lot shipped either earlier or later (21% versus 4%; $P < 0.01$). A linear relationship was observed between the proportion of subjects testing positive for H1N1pdm09 and outdoor temperatures during truck unloading at distributors' central locations. The review of LAIV VE studies showed that in the 2010–2011 and 2013–2014 influenza seasons, no significant effectiveness of LAIV against H1N1pdm09 was demonstrated for the trivalent or quadrivalent formulations of LAIV in the US, respectively, in contrast to significant effectiveness against A/H3N2 and B strains during 2010–2014.

Conclusions: This study showed that the lack of VE observed with LAIV in the US against H1N1pdm09 viruses was associated with exposure of some LAIV lots to temperatures above recommended storage conditions during US distribution, and is likely explained by the increased susceptibility of the A/California/7/2009 (H1N1pdm09) LAIV strain to thermal degradation.

Conclusions: Clinical trial registry: NCT01997450

1. Introduction

Influenza vaccine strain recommendations are updated annually by the World Health Organization and national public health bodies to optimize vaccine effectiveness (VE) in the context of antigenic changes in circulating influenza strains. To monitor the annual effectiveness of influenza vaccines, national public health agencies in the United States (US), Canada, Australia, and Western Europe have supported annual observational studies of VE [1–5] using a prospective, test-negative case control (TNCC) design [6]. In 2013–2014, MedImmune (Gaithersburg, MD), manufacturer of live attenuated influenza vaccine (LAIV, FluMist Quadrivalent™),

Abbreviations: CDC, US Centers for Disease Control and Prevention; E47, glutamic residue at position 47; IIV, inactivated influenza vaccine; LAIV, live attenuated influenza vaccine; SPSN, Sentinel Provider Surveillance Network; TNCC, test-negative case control; US, United States; VE, vaccine effectiveness.

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initiated a TNCC study to evaluate the effectiveness of LAIV among children 2–17 years of age during 4 consecutive influenza seasons to fulfill a regulatory requirement. These TNCC studies are more feasible from operational and ethical standpoints than randomized, placebo-controlled prospective trials. As observational studies, TNCC studies are vulnerable to bias, and small sample sizes can lead to imprecise effectiveness estimates. However, TNCC studies have been shown to provide robust estimates of annual VE and have detected clinically meaningful deficits in vaccine performance [7,8].

In 2013–2014, the TNCC studies conducted by the US Centers for Disease Control and Prevention (CDC) and MedImmune demonstrated no significant VE of LAIV in children during a season dominated by A/H1N1pdm09 strains [9,10]. There was no meaningful circulation of A/H3N2 strains during this season, and in the MedImmune TNCC study, high and statistically significant effectiveness was observed against circulating B strains. The observation of low VE against A/H1N1pdm09 strains was unexpected in light of results of previous randomized controlled trials in children that had demonstrated consistently high efficacy of LAIV against antigenically similar seasonal influenza strains, including seasonal A/H1N1 strains [11], and published results of TNCC studies conducted in 2009–2013 [12–17].

As a live virus vaccine, LAIV is susceptible to heat degradation [18] and it is recommended to be stored and transported at 36–46 °F (2–8 °C) [19]. Additionally, laboratory experiments have demonstrated that the A/California/2009/07 strain had an increased susceptibility to degradation at high temperatures (well above those recommended for storage and handling) due to reduced hemagglutinin stability [20]. As a result, potential exposure to temperatures over the recommended 36–46 °F (2–8 °C) during vaccine shipping and handling became one focus of investigation to explain low VE against A/H1N1pdm09 strains. The current study describes results of post hoc analyses of the MedImmune TNCC study that investigated an association between the lack of LAIV effectiveness against H1N1pdm09 strains and vaccine lot handling conditions during distribution. A comprehensive review of the available data from TNCC and randomized studies describing LAIV VE in children from 2009 to 2010 through 2013–2014 was also conducted to provide additional context regarding potential causes of the lack of LAIV effectiveness against H1N1pmd09 strains in the US in 2013–2014.

2. Materials and methods

The design, methods, and results of the MedImmune TNCC study conducted during the 2013–2014 influenza season have been described previously [9]. For the current analysis, investigational sites provided the lot numbers of LAIV administered to each LAIV recipient enrolled in the study, as well as the vaccine distributors used by the site in the 2013–2014 season.

Most LAIV was shipped at −20 °C by refrigerated trucks from the MedImmune facility in Louisville, Kentucky, to third-party distributors' central locations across the US in Kentucky, Colorado, California, and Tennessee. LAIV doses not shipped by truck, which represented 14% (23/169) of doses included in this analysis, were shipped in insulated parcels that contained dry ice to maintain frozen temperatures during shipping and handling. Examination of US distributor standard operating procedures associated with receipt of truck shipments identified that opportunities existed for exposure of the vaccine to temperatures above the 36–46 °F (2–8 °C) recommended storage temperature at 3 time-points: during unloading of truck vaccine shipments from MedImmune, for up to 2 h following unloading, and for up to 2 h during packing of LAIV for shipment to healthcare professionals. No data by vaccine lot were available to describe temperature exposures during vaccine shipment packing, which occurs within the distribution facility under controlled conditions. However, as truck unloading was confirmed to occur in a setting that was open to outdoor air, the potential temperature exposure at unloading could be estimated based on the outdoor air temperature at the distributor location. The specific date and time of truck unloading was available from bills of lading and FedEx Custom Critical truck monitoring reports for each LAIV lot shipped. The outdoor temperature at the distributors' locations at the nearest hour of LAIV unloading was available from National Oceanic and Atmospheric Administration reports [21].

Consequently, two indicators of LAIV lot shipping conditions could be analyzed in the present study. The first was the calendar date when a vaccine lot was shipped from the MedImmune storage facility to the distributor's central site. The second was the outdoor temperature at the time of truck unloading of the specific vaccine lot. The proportion of LAIV recipients testing positive for H1N1pdm09 strains and LAIV effectiveness were therefore evaluated as a function of these two indicators. Data from unvaccinated children enrolled during the period of H1N1pdm09 strain circulation and LAIV recipients with documented shipping conditions were included in the analysis. LAIV recipients who tested positive for another influenza virus were excluded from the control group in the effectiveness analyses. The proportions of LAIV recipients testing positive for H1N1pdm09 strains were adjusted by logistic regression for each of the following potential confounders in turn: visit date (by month), site, age group (2–4 years, 5–8 years, 9–17 years), vaccination date (before October, during October, after October 2013), and health insurance status (privately insured yes/no). VE (%) was defined as 100 × (1 − adjusted odds ratio), in which the odds ratio represents the odds of exposure to LAIV among influenza cases versus test-negative controls. Adjustment for date of enrollment, site, health insurance status and prior vaccination was conducted with a multivariate logistic regression model. All statistical analyses were conducted with SAS® version 9.3 (SAS Institute, Cary, NC).

To provide additional context and to assist with efforts to identify potential causes for the lack of LAIV effectiveness against H1N1pmd09 strains in the US in 2013–2014, LAIV pediatric effectiveness studies using an observational TNCC design and conducted by the CDC Flu VE Network in the US and the Sentinel Provider Surveillance Network in Canada were reviewed for the time period from the emergence of the H1N1pdm09 strain (i.e., 2009–2010) through 2013–2014. Effectiveness results from these studies were summarized by type/subtype, season, and geographic location.

3. Results

The hourly outdoor temperatures at each distributor's location from July 15 to September 30, 2013 are presented in Fig. 1, with indications for the times when each lot was received and unloaded. As shown in Table 1, the proportion of LAIV recipients who tested positive for H1N1pdm09 was significantly higher among children who received a lot shipped between August 1 and September 15, 2013 compared with children who were administered LAIV from a lot shipped either earlier or later (21% [21/98] and 4% [3/71], respectively; $P < 0.01$). This difference remained significant after adjusting for visit date, site, age group, vaccination date, and health insurance status. Doses shipped between August 1 and September 15, 2013 accounted for 58% (98/169) of LAIV recipients but 87.5% (21/24) of the A/H1N1pdm09 cases detected among LAIV recipients. VE against H1N1pdm09 strains (Table 2) was −32% (95% CI: −149 to 30) for LAIV lots shipped between August 1 and

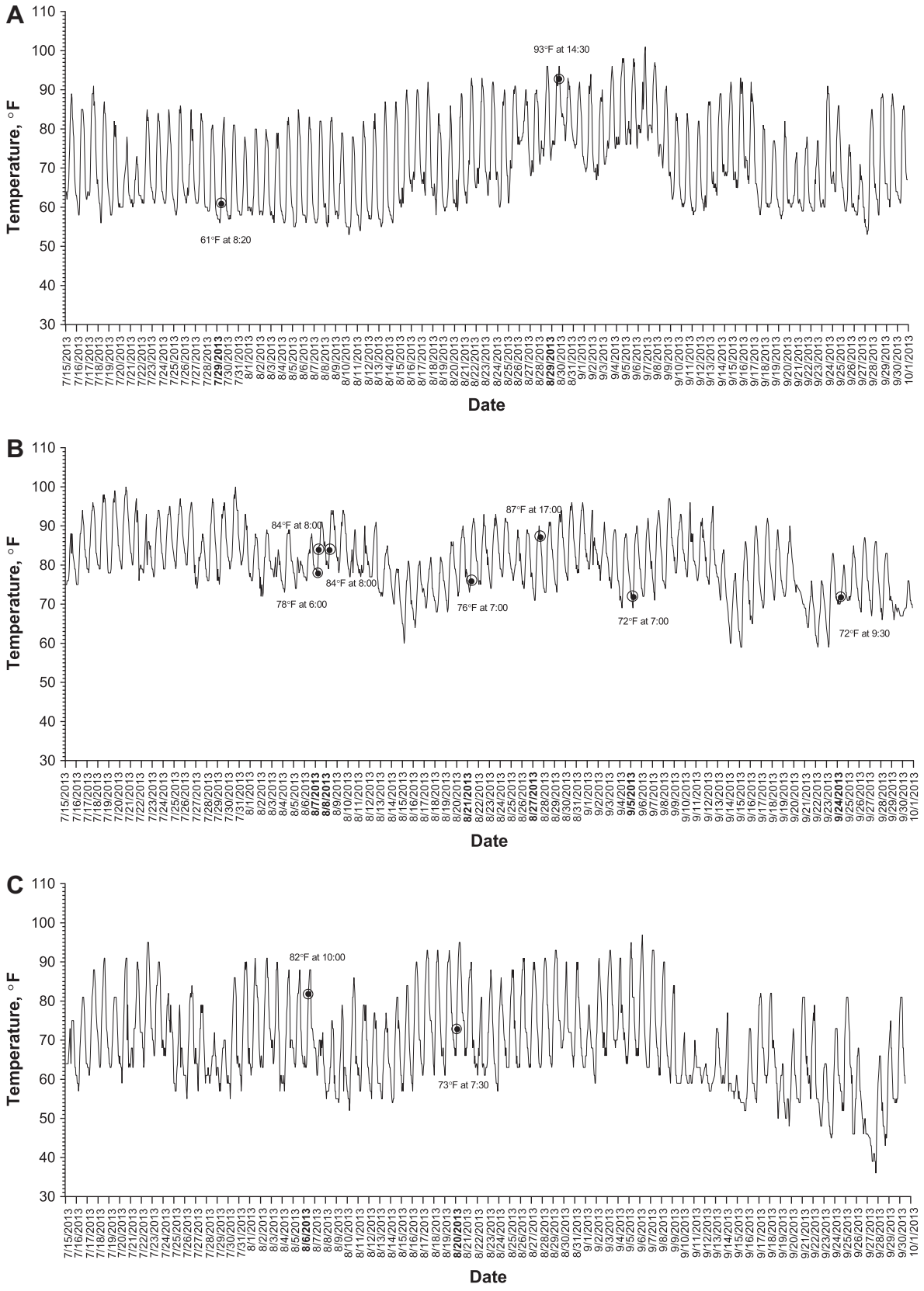

Fig. 1. Hourly outdoor temperature and temperatures during offloading at distributors located in: (A) California, (B) Tennessee, (C) Colorado, and (D) Kentucky.

September 15, 2013 compared with 81% (95% CI: 31 to 95) for LAIV lots shipped either earlier or later; the difference between these two estimates was statistically significant ($P < 0.05$).

The proportions of A/H1N1pdm09 cases among LAIV recipients as a function of the outdoor temperature at the central distributor site at unloading are shown in Fig. 2. A linear relationship was

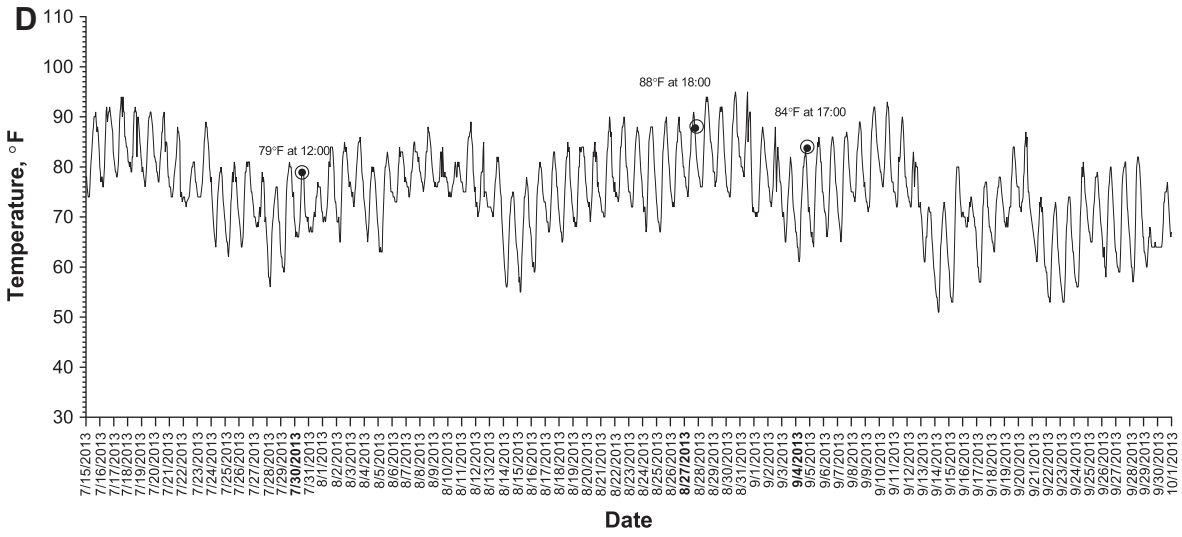

Fig. 1 (*continued*)

Table 1
Proportion of A/H1N1pdm09 cases among LAIV recipients as a function of lot release date.

LAIV recipients enrolled in the study by lot	Shipping date	A/H1N1pdm09 influenza cases
Release before 01 Aug 2013		
BH2029 (n = 32)	26 Jul 2013	3
BH2173 (n = 12)	30 Jul 2013	0
Total (n = 44)		7% (3/44)
Release between 01 Aug 2013 and 15 Sep 2013		
BH2090 (n = 1)	05 Aug 2013	0
BH2091 (n = 3)	06 Aug 2013	1
BH2124 (n = 15)	06 Aug 2013	2
BH2187 (n = 1)	19 Aug 2013	0
BJ2109 (n = 5)	20 Aug 2013	1
BH2186 (n = 23)	27 Aug 2013	5
BJ2013 (n = 11)	27 Aug 2013	4
BJ2154 (n = 34)	04 Sep 2013	6
BJ2014 (n = 5)	04 Sep 2013	2
Total (n = 98)		21% (21/98)
Release after 15 Sep 2013		
BK2019 (n = 16)	16 Sep 2013	0
BK2023 (n = 5)	23 Sep 2013	0
BK2065 (n = 1)	01 Nov 2013	0
BL2049 (n = 2)	08 Nov 2013	0
BL2148 (n = 2)	20 Nov 2013	0
BN2195 (n = 1)	09 Jan 2014	0
Total (n = 27)		0% (0/27)

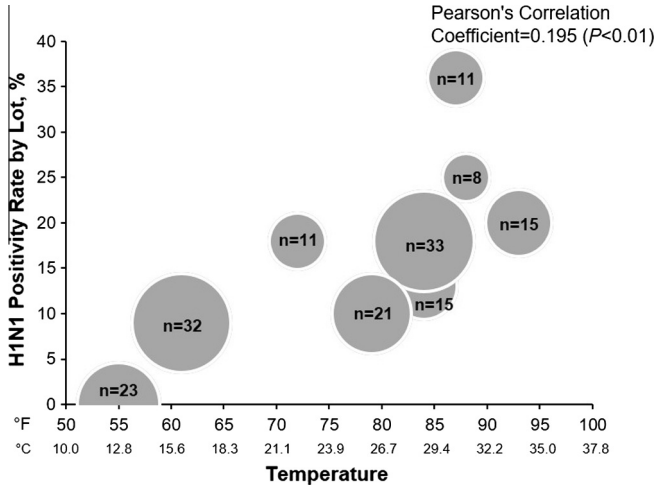

Fig. 2. Association between outdoor temperature at truck unloading and H1N1 illness by lot in LAIV recipients. Circles represent individual lots; circle size is proportional to number of subjects receiving the lot. Lots used in ⩽6 subjects were combined with lots experiencing most similar unloading temperatures. Lots shipped by refrigerated parcel (not by truck) were classified as being exposed to 55 °F (13 °C). LAIV = live attenuated influenza vaccine.

Table 2
LAIV effectiveness estimates against A/H1N1pdm09 as a function of shipping conditions in 2013–2014.

	Unvaccinated subjects n = 312	LAIV recipients (n = 165[a])			
		Lots shipped in August and early September, 2013		Lots exposed to temperature ⩾84 °F (29 °C)	
		Yes	No	Yes	No
Cases	68	21	3	17	7
Controls	244	75	66	63	78
Crude effectiveness		−1 (−75 to 42)[c]	84 (46 to 95)[c]	3 (−76 to 47)[c]	68 (27 to 86)[c]
Adjusted effectiveness[b]		−32 (−149 to 30)[c]	81 (31 to 95)[c]	−30 (−158 to 35)[c]	60 (−1 to 84)[c]

[a] Four LAIV recipients who tested positive for another influenza strain were excluded from this analysis.
[b] Adjustment on date of enrollment, site, health insurance status, and prior vaccination.
[c] 95% confidence interval.

observed, with an increasing proportion of subjects testing positive for H1N1pdm09 with increasing temperatures at truck unloading of the vaccine (Pearson's correlation coefficient = 0.195; $P = 0.01$). The study population was balanced between LAIV recipients who received vaccine from lots that were potentially exposed to outdoor temperature ⩾84 °F (29 °C) or not: n = 82 and n = 87, respectively. The proportion of LAIV recipients who tested positive for A/H1N1pdm09 was significantly higher when the ambient temperature was ⩾84 °F (21% [17/82] versus 8% [7/87]; $P = 0.02$). This difference remained significant after adjusting for site, age group, vaccination date, and health insurance status. VE against the H1N1pdm09 strain (Table 2) was 60% (95% CI: −1 to 84) for unexposed LAIV lots compared with −30% (95% CI: −158 to 35) for LAIV lots unloaded in outdoor temperature ⩾84 °F (29 °C); the difference between these two estimates was statistically significant ($P < 0.05$).

Fig. 3 depicts VE estimates against circulating A/H3N2, B and A/H1N1pdm09 strains from TNCC studies conducted in the US and Canada from 2009 to 2010 through 2013–2014 in children ⩽9 years of age, as results were consistently available across studies in this age subgroup [9,22,23]. LAIV was consistently effective against H3N2 and B strains in all study seasons. When administered as a monovalent formulation in the US during the 2009–2010 season, LAIV was effective against H1N1pdm09 strains when cases were assessed beginning 7 days after vaccination, with a VE of 82% (95% CI: 14 to 96) [14]. In the 2010–2011 and 2013–2014 influenza seasons, no significant effectiveness of LAIV against H1N1pdm09 in the US was demonstrated for the trivalent or quadrivalent formulations of the vaccine, respectively, in contrast to the high effectiveness demonstrated against A/H3N2 and B strains during those same seasons [9,10,22,24,25]. However, LAIV was effective against H1N1pdm09 when administered as a trivalent formulation in the 2013–2014 season in Canada, with a VE of 85% (95% CI: −22 to 98) in children 2–8 years of age and 87% (95% CI: 2 to 98) in children and adolescents 2–19 years of age in British Columbia, Alberta, and Quebec [23]. Further evidence that LAIV was effective in Canada during the 2013–2104 season was provided by a cluster-randomized trial among elementary school children in Ontario, during which 95% of influenza cases were due to A/H1N1pdm09 strains [26]. The incidence of influenza among children vaccinated with LAIV was 0.13 per 1000 person-days compared with 1.24 per 1000 person-days for children vaccinated with inactivated influenza vaccine (IIV); ($P < 0.05$). Overall, the review of available LAIV VE estimates from 2009 to 2010 through 2013–2014 demonstrated that the lack of LAIV effectiveness was limited to A/H1N1pdm09 strains and to US studies conducted in 2010–2011 and 2013–2014.

4. Discussion

These analyses suggest that the known increased susceptibility of the A/California/7/2009pdm09 LAIV strain to thermal degradation and potential exposure of some LAIV lots to heat during US distribution may explain the lack of VE observed with LAIV in 2013–2014 in the US. LAIV effectiveness against A/H1N1pdm09 strains was significantly associated with both vaccine shipping date and the outdoor temperatures to which lots could have been exposed during US distribution. LAIV lots demonstrated reduced effectiveness when shipped between August 1 and September 15 or when unloaded at distributors at a time when outdoor temperatures were ⩾84 °F (29 °C). However, LAIV was significantly effective when the lots were shipped before August or after mid-September 2013, and LAIV effectiveness was very close to

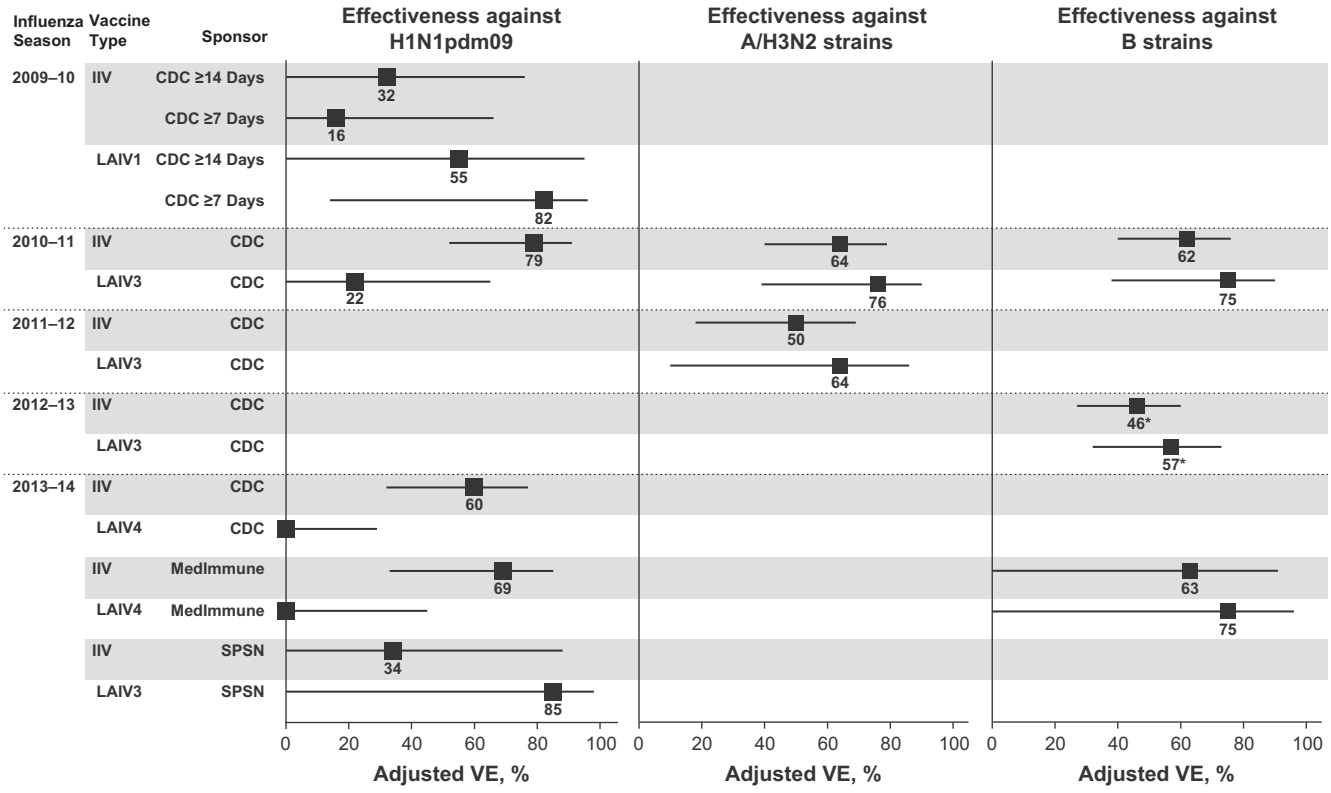

Fig. 3. Vaccine effectiveness against H1N1pdm09, H3N2, and B strains in children since 2009 through 2013–2014. The grayed background rows represent IIV. *The majority of identified strains in the 2012–2013 CDC study in children aged 8 years or less were B strains, but there was also a large proportion of A/H3N2 strains. IIV = inactivated influenza vaccine; LAIV = live attenuated influenza vaccine; SPSN = Sentinel Provider Surveillance Network (data collected in Alberta, British Columbia and Quebec).

statistical significance when it was unloaded at distributors where outdoor temperatures were <84 °F.

A review of data since the 2009 influenza A/H1N1pdm09 pandemic indicated that LAIV was similarly not effective against A/H1N1pdm09 strains in the 2010–2011 season in the US, when most LAIV doses were shipped before mid-September. In contrast, significant effectiveness was observed in the US in 2009–2010 when LAIV was shipped exclusively after mid-September, and in 2013–2014 in Canada when LAIV was shipped starting in November and where outdoor temperatures are typically lower than in the US. The conclusion that some LAIV lots were effective in the US in 2013–2014 is also supported by the trend of effectiveness observed in a US household-based cohort study [27].

The association between reduced effectiveness against H1N1pdm09 in the US and potential heat exposure during LAIV distribution is supported by laboratory studies that documented reduced thermal stability of A/California/7/2009pdm09. Specifically, a glutamic acid (E) residue at position 47 (E47) of the hemagglutinin stalk that was present in early H1N1pdm09 wild-type strains, including the A/California/7/2009 strain, destabilizes the hemagglutinin trimer, rendering it more vulnerable to inactivation upon exposure to extreme heat or acid and reducing its infectivity in ferrets [20]. Additionally, the hemagglutinin stalk E47 sequence may have reduced the viral fitness of early H1N1pdm09 strains as contemporary H1N1pdm09 strains have evolved to contain a lysine at position 47, which increases hemagglutinin stability [20]. When these laboratory studies were first conducted, they did not appear to have any clinical significance, given the demonstration of high effectiveness of the A/California/7/2009 LAIV strain in US children in the 2009–2010 season. However, the observed reduced thermal stability is consistent with the hypothesis that the A/California/7/2009 LAIV strain may have been compromised by heat exposure during US distribution in 2010–2011 and 2013–2014 when LAIV was distributed during warmer months, resulting in reduced vaccine potency at the time of administration and thus reduced effectiveness. A previous study demonstrated substantially reduced efficacy of LAIV when administered at a potency of 10^6 viral particles compared with the standard LAIV potency of 10^7 [28]. Multiple clinical studies in children have demonstrated vaccine virus replication and immunologic responses with the A/California/07/2009 LAIV strain [29–32], providing evidence that the A/California/7/2009 LAIV strain was biologically active in humans, capable of replication, and able to induce relevant strain-specific immunity when administered at full potency.

These observed associations do not prove a causal link. It is possible that there is an alternative causal factor or factors that correlate with these findings. Additionally, the precise extent to which vaccine lots may have exceeded their recommended storage temperatures of 36–46 °F (2–8 °C) is unknown because there was no direct monitoring of vaccine lot temperatures after receipt by distributors. However, following an extensive investigation into other potential causes of the observed low LAIV VE in 2013–2014, no other potential explanations have been identified that are consistent with the available data regarding LAIV VE in recent seasons. A comprehensive investigation of LAIV manufacturing and release testing was conducted. Additionally, various potential explanations for decreased vaccine effectiveness previously described in randomized controlled trials or case-control observational studies were evaluated. Specifically, low effectiveness of IIV has been previously associated with prior vaccination, poor antigenic match between vaccine and circulating strains, age $\geqslant$65 years, or waning protection during the influenza season [7,16,33–39]. However, no findings related to manufacturing, testing, prior vaccination, vaccine strain mismatch, or waning protection were identified that might explain the lack of effectiveness of LAIV during the 2013–2014 season (data not shown). The hypothesis that viral interference between the four LAIV vaccine strains could explain the absence of effectiveness against H1N1pdm09 strains is also not consistent with the available clinical evidence [10,14,22,23]. In particular, with the observations of significant variability by vaccine lot or the observation that the trivalent formulation was effective against A/H1N1pdm09 strains in Canada in 2013–2014 but not in the US in 2010–2011.

Previous analyses of the TNCC studies conducted by the CDC and MedImmune during the 2013–2014 season investigated whether age or prior vaccination might be associated with the low effectiveness against A/H1N1pdm09 strains [9,10,22]. In both studies, VE trended higher in older children compared with younger children. Additionally, data on LAIV VE available from other TNCC and randomized studies demonstrated that 2009–2014 trivalent and quadrivalent LAIV formulations were consistently effective in children against circulating H3N2 and B strains [14,37,40]. These observations are not consistent with the hypothesis that preexisting immunity due to more natural influenza infections or more years of annual vaccination with increasing age of US children were responsible for the lack of LAIV VE. On the contrary, the trend toward higher VE in older children observed in the CDC and MedImmune studies in 2013–2014 would be consistent with the ability of a reduced-potency LAIV to boost existing immunity in children previously infected with wild-type H1N1pdm09 influenza.

In summary, this investigation showed that the lack of VE observed with LAIV in the US against H1N1pdm09 viruses was associated with exposure of some LAIV lots to temperatures above the recommended storage conditions of 36–46 °F (2–8 °C) during US distribution, and is likely explained by the increased susceptibility of the A/California/7/2009 (H1N1pdm09) LAIV strain to thermal degradation. Other recent LAIV strains without this susceptibility to thermal degradation appear to have been unaffected by heat exposures during US distribution in recent seasons. Nevertheless, our findings reinforce the need for adherence to recommended storage temperatures throughout the entire vaccine distribution chain. To ensure effectiveness against H1N1pdm09 strains in future seasons, the A/California H1N1pdm09 LAIV strain has been replaced in the 2015–2016 LAIV formulation with an antigenically equivalent A/H1N1pdm09 vaccine strain (A/Bolivia/559/2013) that does not possess the E47 residue in the hemagglutinin stalk and is more stable.

Conflicts of interest

KLC is a former employee of AstraZeneca. HC, RMM, and CSA are employees of AstraZeneca.

Author contributions

All authors participated in the development of this article. CSA and HC contributed to study design. HC performed all data analyses. KLC prepared the manuscript first draft. CSA, HC, RMM, and KLC provided evaluation of data and critical review of the manuscript.

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