PERSONAL VIEW ARTICLE

Urgent challenges in implementing live attenuated influenza vaccine

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Word count unstructured summary: 177

Word count manuscript: 3000

Summary

Conflicting reports have emerged about the effectiveness of the live attenuated influenza vaccine (LAIV). LAIV appears to be protecting particularly poorly against currently circulating H1N1 viruses that are derived from the 2009 pandemic H1N1 (pH1N1) viruses. During the 2015/16 influenza season, when pH1N1 was the predominant virus, studies from the United States (US) reported a complete lack of effectiveness of LAIV in children. This led to a critical decision in the US to recommend that LAIV not be used in 2016/17 and to switch to the inactivated influenza vaccine. Other countries, including the UK, Canada and Finland, have continued to recommend use of LAIV. This policy divergence and uncertainty has far reaching implications for the entire global community, given the importance of LAIV production capability for pandemic preparedness. In this Personal View, we discuss possible explanations for the observed reduced effectiveness of LAIV and highlight underpinning scientific questions. Further research to understand the reasons for these observations is essential to enable informed public health policy and commercial decisions about vaccine production and development in coming years.

Introduction

Influenza vaccination remains the main strategy to control the burden of seasonal influenza disease that affects 5-10% of adults and 20-30% of children, resulting in 250,000-500,000 deaths worldwide each year.[1,2] In the US, 140 million doses of vaccine are distributed each year and vaccination is estimated to prevent nearly a quarter of predicted influenza-associated deaths.[3] Vaccination is also the primary public health response to a global influenza pandemic.

Influenza vaccines contain a mixture of three or four components designed to protect against the different viruses that circulate contemporaneously as seasonal influenza viruses. Currently, these are two influenza A viruses (H1N1 and H3N2 subtypes) and two influenza B viruses (Victoria and Yamagata lineages). Vaccine components are reviewed and updated regularly by the WHO in line with observed antigenic drift of influenza viruses.

The traditional inactivated influenza vaccine (IIV) was introduced in the 1940s. IIV is administered by intramuscular/intradermal injection, is licensed for use in all ages and has a good safety record. However, effectiveness rates vary, averaging around 50-60%.[4,5] More recently, live attenuated influenza vaccine (LAIV) was demonstrated to have greater efficacy than IIV in children, with absolute efficacy rates of 75-80%.[6–8]

Internal genes of LAIV viruses are derived from a cold-adapted, attenuated strain of virus, either Ann Arbor/1960 (from the US) or Leningrad/1957 (from Russia). Haemagglutinin (HA) and neuraminidase (NA) surface antigens are engineered to be representative of currently circulating strains. LAIV is nasally administered and vaccine viruses replicate only in the air-cooled environment of the human upper respiratory tract. This results in a mild and self-limiting infection; virus cannot replicate at the warmer temperature of the lower respiratory tract. Yet, the immune response stimulated is robust and unlike IIV includes cellular, humoral and mucosal responses.[9–11]

The “Leningrad” LAIV has been used widely in Russia for over 50 years.[12] The “Ann Arbor” product became licensed in the US in 2003 and is available for 2-49 year olds, in Canada since 2010 for 2-59 year olds, and in the European Union since 2011 for 2-17 year olds. LAIV was introduced to childhood vaccination programmes in the UK in 2013 and Finland in 2015. Despite official recommendation and promotion of seasonal influenza vaccination, uptake rates in eligible individuals vary between countries.[13,14]

In this Personal View, we aim to discuss possible explanations for divergent observations about the performance of LAIV and highlight important scientific questions. Focussed efforts to develop an evidence base to resolve uncertainties will aid timely clinical, commercial and policy decisions about use of LAIV.

Recent observations of reduced effectiveness of LAIV

Before licensure, vaccine efficacy is assessed in randomised controlled trials (RCTs). This demonstrates the "best case scenario" of a vaccine's protective effect under controlled conditions in carefully selected populations. Post licensure, vaccine effectiveness (VE) is monitored under "real world" conditions in observational studies. Currently, the most widely used study design to calculate influenza VE is the test-negative design (TND), utilising routinely collected surveillance data from subjects with influenza-like illness (ILI). Participants with a positive influenza test are assigned as "cases" and those who test negative are "controls". Vaccination history is collated and VE calculated as [1 – odds ratio] x100. The final numbers released as percentage effectiveness are the result of further adjustments that account for age, timing, and geography.

Effectiveness of LAIV in the US is reported to have declined since 2009, and the H1N1 component appears to be the worst affected.[15,16] In RCTs from the early 2000s, LAIV was highly efficacious (~85%) against seasonal H1N1 strains in children.[7] In 2009, a novel swine-origin pandemic H1N1 (pH1N1) virus emerged and replaced the seasonal H1N1 strain that had been circulating in humans since 1977. In subsequent years when pH1N1 circulated as the predominant seasonal virus, the US documented low pH1N1 VE: 15% in 2010/11, 17% in 2013/14 and -21% in 2015/16.[16,17] As a consequence, the US Advisory Committee for Immunization Practices (ACIP) recommended that LAIV not be used in the US in 2016/17 and that IIV should be utilized instead.[18]

In the UK, Canada and Finland, the problem with the pH1N1 component although still evident, was not nearly as drastic, with effectiveness rates of 40-50% reported in 2015/16.[19–21] Emphasising the variability in VE results, the US ICICLE study also reported pH1N1 VE of 50%.[22] Importantly, VE estimates against pH1N1 for IIV have been consistently higher than LAIV in all countries (Table 1) and closer to that reported for H3N2 and influenza B. In contrast to the US, the UK, Canada and Finland continued to recommend LAIV for the 2016/17 season.[23-25]

The recent introduction of LAIV in the UK and Finland was driven by cost-effectiveness studies demonstrating that controlling influenza in children can have protective effects on the wider community, including more vulnerable groups.[26,27] Pebody et al. estimated that vaccinating 16 UK primary school children could prevent one primary care ILI consultation and vaccinating 317 children could prevent one influenza hospitalisation.[28] Similar herd protective effects from vaccinating school children have been demonstrated in Russia,[29] Japan,[30] and the US.[31,32] These beneficial effects for those outside the direct programme also contribute to national policy decisions on influenza vaccination.[33]

LAIV has been highlighted as a particularly valuable technology given that the straightforward, high-yield manufacturing process and needle-free administration are more transferable to low- and middle- income countries and advantageous for pandemic surge production.[34] There are very few studies of LAIV in such populations but two recent RCTs assessed efficacy of "Leningrad" LAIV in Senegal[35] and Bangladesh[36]. Surprisingly, the results from these two

studies are very different, with no efficacy demonstrated in Senegal, but 41% efficacy in Bangladesh (Table 2). The reasons for these discrepant results are unclear, but could be due to differences in the predominant virus circulating (more pH1N1 in Senegal that year), population characteristics or environmental factors. Understanding the contributions of these factors is important, given the critical role of LAIV in the WHO's Global Action Plan for Influenza Vaccines, which strives to expand influenza vaccine manufacturing capabilities in developing countries as a strategy to improve global pandemic preparedness.[37]

Hypotheses to explain the reduced effectiveness of LAIV

The '"real world" effectiveness of LAIV in observational studies has varied substantially and has not matched the potency demonstrated in RCTs performed before 2009, when seasonal H1N1 viruses circulated. A clear global trend can be seen of reduced effectiveness of the pH1N1 component of LAIV in particular, which is under-performing compared to IIV in all studies. The main hypotheses to explain these observations include: (i) heterogeneity and bias associated with observational studies used to calculate VE; (ii) population characteristics and levels of pre-existing immunity that may explain divergent reports from different countries; (iii) biological characteristics of the pH1N1 virus that can explain the poor performance of this vaccine component. Focussed research in each of these areas is needed, some urgently so, to optimise and maintain the use of LAIV globally.

(i)Variability between observational studies used to calculate influenza VE

The TND study design, using routine surveillance data, has been widely applied for VE monitoring because it is convenient and cost-effective. Accuracy and validity of the TND design has been demonstrated,[38] although inherent bias and confounding factors still exist in this observational study design. In practice, small sample sizes leading to wide confidence intervals and lack of statistical power, particularly upon sample stratification, contribute to imprecise VE estimates. It is important to interpret %VE figures with consideration of their confidence limits. Furthermore, variability in study methodology and analytical techniques that exist between countries may limit comparability of data.[39,40] For example, differences in inclusion criteria, study setting, influenza sampling type and quality and how confounding factors are adjusted for. That said, VE estimates for IIV, which would be affected by similar factors, have remained generally consistent across studies. As more experience is gained on the use and pitfalls of TND studies, with increased pooling of international data, there is greater opportunity for harmonisation of protocols.[41]

(ii)Prior vaccination and immunity

In pre-licensure RCTs, LAIV was not demonstrated to be any more efficacious than IIV in adults. Absolute efficacy in 18-49 year olds was lower than in children and efficacy was not demonstrated in the over 50s, leading to preferential recommendation for LAIV in children.[6,42] The common interpretation of this finding is that adults have prior immune history of influenza infections and

possess cellular immunity to conserved internal viral proteins that can supress local mucosal replication of LAIV well enough to preclude a robust immune response. This cross-reactive immunity may be what protects many adults from severe illness during a pandemic.[43]

Between 2008 and 2014, uptake of LAIV in US 2-8 year olds increased from 20.1% to 38%.[44] One suggestion is that VE observations in the US are a result of recurrent exposure to LAIV over a prolonged time. Indeed, US children who receive annual LAIV have effectively been repeatedly infected with a mild influenza for the last decade, every year boosting their heterologous (cross-reactive) immunity. Considering that the lifetime experience of influenza is estimated around one natural infection every 5 years,[45] administering LAIV annually represents a significant increase on natural frequency. In this sense, US children who receive annual LAIV have perhaps become immunologically akin to adults, in whom the attenuated vaccine viruses cannot replicate effectively. Support for this hypothesis might come from age stratified data, in which one might expect older children to be less well protected; however, current observational data sets are too small to allow firm conclusions to be drawn.

Given that annual vaccination with LAIV is planned for children in several countries in coming years, it is important to understand whether repeated vaccination over a longer term has contributed to the current difficulty using LAIV in the US. Although some studies have indicated that a repeat dose of LAIV in two consecutive years does not diminish efficacy,[46] the consequence of repeating vaccination every year for a decade has not been explored. Whilst studies do support the notion that viral shedding and immune response to LAIV may decline upon repeated use[47,48] and with increasing age,[49] this has not yet been correlated to a reduction in vaccine efficacy.[50]

Moreover, use of live vaccine on an annual basis is unique to influenza and further study is required to understand the implications. For example, whether subtle changes in antigenicity affect LAIV "take" more than IIV, requiring more frequent strain updates or different methods of strain selection than that established for IIV. Additionally, to account for non-specific effects from frequent administration of a live vaccine, that may be quite distinct from inactivated vaccines.[51]

(iii)Biological characteristics of the pH1N1 virus

(a)Reduced infectivity of the pH1N1 component

Added to this is the unusual circumstance of pH1N1 being a virus only recently introduced into human circulation. Some key adaptive changes required of zoonotic influenza viruses to sustain transmission in humans occur in the HA gene, the major viral antigen. These mutations enhance the replicative ability of virus in the upper respiratory tract, including adaption to bind sialic acid receptors that are abundant in human airway and to increase the pH/temperature stability of the HA protein, allowing virus to survive the mildly acidic environment of the human nasal mucosa.[52] By summer 2009, the

emerging pH1N1 virus had achieved the minimal requirements to overcome the initial host range barriers, but was far from a fully human-adapted virus. Over the next few years pH1N1 has accumulated further 'humanizing' mutations and become more acid stable,[53,54] but the virus is still not as fine-tuned to its new host as the human-specific influenza B viruses or H3N2 viruses that have circulated in humans since 1968. Thus, immunogenicity of LAIV with pH1N1 HA and NA antigens might be lower than for the other vaccine components because replication in the target tissue is lower.

Some evidence to support this does exist although more focussed studies are urgently needed. Influenza viruses with unstable HA replicate less well in the upper respiratory tract of ferrets, the most representative animal model.[54] A stabilising mutation present in more recent pH1N1 isolates but not the earliest strains from 2009 was associated with greater infectivity in the ferret nasal tract.[55] O'Donnell et al.[56] demonstrated that viruses reassorted to contain "Ann Arbor" internal genes became less pH stable than their wild-type counterparts due to cold-adapting mutations in the viral M gene. This reduction in stability could have greater impact on infectivity of pH1N1, which may already exist closer to the threshold for inactivation in the upper respiratory tract. Moreover, in the study from Senegal, Leningrad LAIV pH1N1 virus was shed by vaccinees less frequently than H3N2 and B vaccine components.[35] More quantitative information on shedding would help to assess the relative fitness of vaccine viruses. The lack of a clear correlate of protection for LAIV impairs our understanding of immunogenicity; however, a Norwegian study demonstrated that children vaccinated with LAIV had significant haemagglutination inhibition titre and salivary IgA against H3N2 and B viruses but not for pH1N1.[11]

The extent of attenuation in internal viral genes could be key to take rate in different age groups. In Russia, a more attenuated viral strain (47x passaged Leningrad/1957) was developed for use in children, where as a lower passage variant is used in adults.[57] Ann Arbor/1960 was observed to be more attenuated than both Leningrad/1957 strains in primary human bronchial epithelial cells[58] and less immunogenic in mice.[59] Achieving the correct balance of attenuation versus fitness requires further understanding and internal gene backbones may need to be customised to the viral surface antigens, or the target patient group.

(b)Reduced thermostability of the pH1N1 component

A further concern is that the unstable HA of the pH1N1 component may have been sensitive to breaks in the cold chain, which could have contributed to poor effectiveness between 2010 and 2014.[60] In response to this, vaccine manufacturers took the initiative to update the pH1N1 component of LAIV in their 2015/16 product to include the HA from a more recent pH1N1 isolate (A/Bolivia/559/2013) that contains a known HA stabilising mutation[55]. In addition, measures were taken to improve maintenance of the cold chain. However, this did not solve the problem, as 2015/16 was again a year with poor VE recorded. Improved understanding of the viral genetic basis for good infectivity in the nasal tract and stability during vaccine distribution would improve the ability to select appropriate strains in the future.

(c)Viral interference between live vaccine components

Viral interference occurs when infection with one virus impedes infection with another virus in the same host. For successful protection against all components in a multivalent live vaccine, adequate replication of each individual vaccine strain is required. As part of a multivalent product, it may be that the pH1N1 component is struggling to compete with other influenza viruses that are better adapted for the host cell target. If correct, this situation may have been exacerbated in 2013 when vaccine valency was increased from three to four strains, to include both Victoria and Yamagata influenza B lineages that have co-circulated in recent times. However, given that the pH1N1 component fared poorly in 2010/11 when the trivalent formulation was used, a switch to quadrivalent vaccine could only have made an existent problem worse. Further studies, for example in the ferret model, would help to understand mechanisms and impact of viral interference.[61] A parallel experience with live attenuated polio vaccine, where type one and three viruses were poorly immunogenic in trivalent vaccine, was rectified by modifying the relative doses of each serotype to a 10:1:3 formulation.[62] Approaches such as this, to facilitate the pH1N1 virus to better compete, should be a focus for future research.

Conclusion

The current situation is potentially a perfect storm of: (i)a set of vaccinees with enhanced pre-existing immunity, (ii)a vaccine virus (pH1N1) that is not optimally adapted for replication in the human nose, (iii)inclusion of multiple strains of virus into the vaccine, with which the weakest component needs to compete. The underlying biological root causes associated with poor performance may be difficult to tease out in observational studies, which are themselves subject to significant variation.

LAIV has been introduced with great promise and many advantageous features, yet there remain many unknowns: how LAIV can work in different communities with different exposures to natural and vaccine antigens, what are the correlates of protection, whether annual dosing with LAIV is required to maintain and update immunity in the same way as IIV, how to select and engineer vaccine strains with optimal replication in the nasal tract, and how to accurately monitor vaccine effectiveness. LAIV plays a key role in the WHO's pandemic preparedness plans and is currently one of the leading technologies that would aid in universal access to vaccine in the face of a severe influenza pandemic. Understanding how to make and use LAIV that protects against emerging influenza viruses should therefore be a public health priority. Diminishing seasonal LAIV production capacity would have serious impact on our ability to produce adequate quantities of pandemic vaccine.[63] Given that the US is the world's largest market for seasonal influenza vaccine, the ACIP recommendation for 2016/17 has wide-reaching implications for the entire global community, particularly in view of the concern that Ann Arbor LAIV may not remain commercially viable. Urgent action is required to overcome uncertainties about

LAIV effectiveness to enable public health policy and commercial decisions to be made for forthcoming years across the world.

Of further concern is that some scientists advise against turning to IIV for very young children.[64,65] When children experience their first influenza infection the clinical outcome can be severe,[66] which may partly be due to a lack of cross-protective cellular immunity. Vaccinating children every year with IIV that induces a sterilizing immune response will limit acquisition of cellular immunity through natural exposures. In the face of a new strain emerging for which there is vaccine mismatch, for example a significant drift or pandemic, these young individuals could be at risk of more severe disease[43] than if they had developed cross-protective immunity through prior natural infections or vaccination with LAIV. Therefore, it is imperative we understand how to use LAIV properly in children.

Contributors
AS and WB wrote the first draft of the article. AS performed the literature search, designed the tables and panel and expanded the article. All authors made contributions to discussions on the scope of the article, critically reviewed revisions and the final manuscript.

Declarations of interest
All authors declare no competing interests

Search strategy and selection criteria

We searched PubMed and Google Scholar databases using search terms "influenza" AND "vaccin*" AND ("live" OR "attenuated" OR "cold adapted" OR "Ann Arbor" OR "Leningrad" OR "efficacy" OR "effectiveness"), "LAIV", "CAIV", "live attenuated versus inactivated" for English language articles published up to and including April, 2017. Relevant articles and presentations to scientific conferences or public health bodies were identified through searches of publically available information from the WHO and country-specific public health websites (e.g. US CDC, UK PHE). Original articles, review articles, editorials and commentaries identified from these searches were reviewed and relevant references cited in those articles examined.

Panel

Priority areas for research to inform clinical and policy decisions about LAIV

Studies to understand and improve the pandemic H1N1 vaccine component in humans

Questions to be addressed:
What are the kinetics of vaccine virus replication and shedding in LAIV recipients?
What viral characteristics are desirable in selecting LAIV strains and how can they be identified?
What genetic changes could be engineered into vaccine viruses to optimise replication?
What is the impact of viral interference between LAIV components and how can this be minimised?
What strategies can be employed to balance the replication and immunogenicity of individual components?
What are the differences between Ann Arbor and Leningrad donor viruses that may aid our understanding about optimising LAIV immunogenicity?

Studies to understand the impact of pre-existing immunity and prior vaccination history on vaccine effectiveness

Questions to be addressed:
What is the impact of long-term exposure to LAIV on its effectiveness?
What is the durability of immune protection from LAIV? Is annual dosing with LAIV required to maintain immunity?
Do subtle changes in antigenicity affect LAIV "take" more that IIV, perhaps requiring more frequent strain updates or different methods of strain selection than those established for IIV?
What are the relative contributions of cellular, humoral and mucosal immune responses induced by LAIV?
What are the correlates of protection for LAIV?
How do population characteristics, gender, nutritional status and environmental factors contribute to LAIV effectiveness?
What are the public health benefits of LAIV versus IIV, for example conferring herd protection and non-specific beneficial effects?

Improving monitoring strategies for influenza vaccine effectiveness

Questions to be addressed:
What is the optimum study design to measure influenza vaccine effectiveness?
Can internationally recognised protocols and methods for statistical analysis be developed to standardise and compare vaccine effectiveness monitoring studies?

Country of origin	Study type	Participant ages (years)	Ref	LAIV				IIV			
				All strains		pH1N1		All strains		pH1N1	
				Adjusted [Crude] VE (%)	95% CI	Adjusted [Crude] VE (%)	95% CI	Adjusted [Crude] VE (%)	95% CI	Adjusted [Crude] VE (%)	95% CI
US (CDC)	TND	2-17	17,18,67	3	-49 to 37	-21 [-49]	-108 to 30	63	52 to 72**	65 [57]	50 to 75**
US (DoD)	TND	2-17	17,67	53	25 to 70**	15	-48 to 51	66	50 to 75**	68	45 to 80**
US (ICICLE)	TND	2-17	22,67	46	7 to 69	50	-2 to 75	65	48 to 76	71	50 to 80**
UK	TND	2-17	20	58 [45]	25 to 76 [12 to 65]	42 [11]	-9 to 69 [-48 to 47]	78 [64]	7 to 95 [-23 to 90]	100 [100]	13 to 100† [13 to 100]
Canada	TND	2-17	19	ne [74]	[35 to 90]**	ne [51]	[-40 to 80]**	ne [63]	[25 to 80]**	ne [87]	[40 to 95]**
Finland	Cohort	2	21	51 [47]	28 to 66 [23 to 63]	48* [45]	22 to 65 [18 to 64]	61 [58]	31 to 78 [26 to 77]	80* [78]	50 to 92 [47 to 91]

Table 1. Live attenuated and inactivated influenza vaccine effectiveness reported in studies performed in 2015/16

Abbreviations: VE = vaccine effectiveness; 95% CI = 95% confidence interval; TND = test negative design; pH1N1 = 2009 pandemic H1N1 virus; CDC=Centres for Disease Control and Prevention; DoD=Department of Defence; ICICLE=Influenza Clinical Investigation for Children; LAIV=live attenuated influenza vaccine; IIV=inactivated influenza vaccine; Ref = reference; ne = not estimated due to small sample size

*Data shown for all influenza A from Finland

**Confidence interval estimated from figure

† Cornfield's unadjusted estimate

Data provided where available

Country of origin	LAIV type	Study type	Participant ages (years)	Predominant circulating viruses	Ref	LAIV			
						All strains		pH1N1	
						Adjusted VE (%)	95% CI	Adjusted VE (%)	95% CI
US (CDC)	Ann Arbor	TND	2-17	pH1N1, B-Yamagata	[68,16]	-1	-40 to 40**	17	-39 to 51
US (ICICLE)	Ann Arbor	TND	2-17	pH1N1, B-Yamagata	[69]	32	-13 to 59	13	-5 to 51
US (HIVE)	Ann Arbor	Cohort	2-8 9-17	pH1N1	[70]			82 11	-65 to 98 -658 to 90
Canada (SPSN)	Ann Arbor	TND	2-19	pH1N1, B-Yamagata	[71]	83*	25 to 96	86*	-11 to 98
Senegal	Leningrad	RCT	2-5	pH1N1, B-Victoria	[35]	0	-26 to 21	-9.7	-63 to 26
Bangladesh	Leningrad	RCT	2-4	H3N2, pH1N1, B-Victoria	[36]	41	28 to 52	50	9 to 73

Table 2. Live attenuated influenza vaccine effectiveness reported in studies performed in 2013/14

Abbreviations: VE = vaccine effectiveness; 95% CI = 95% confidence interval; TND = test negative design; RCT=randomised controlled trial; CDC=Centres for Disease Control and Prevention; ICICLE=Influenza Clinical Investigation for Children; HIVE=Household Influenza Vaccine Effectiveness; SPSN=Sentinel Physician Surveillance Network; LAIV=live attenuated influenza vaccine; Ref=reference

*Unadjusted VE provided (adjusted VE not estimated due to small sample size)

** Confidence interval estimated from figure

Data provided where available

No data for US (HIVE) “all strains” due to predominance of pH1N1 in study cohort

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