The impact of candidate influenza virus and egg-based manufacture on vaccine effectiveness: Literature review and expert consensus

Sankarasubramanian Rajaram a,⇑, Radek Wojcik b, Catherine Moore c, Raúl Ortiz de Lejarazu d, Simon de Lusignan e,f, Emanuele Montomoli g, Alessandro Rossi h, Alberto Pérez-Rubio i, Antoni Trilla j, Vincenzo Baldo k, Ravi Jandhyala b, George Kassianos l

a Seqirus, Maidenhead, UK
b Medialis Ltd, Banbury, UK
c Wales Specialist Virology Centre, Public Health Wales, Cardiff, UK
d Valladolid National Influenza Centre, Valladolid, Spain
e Nuffield Department of Primary Care Health Sciences, University of Oxford, Oxford, UK
f Royal College of General Practitioners Research and Surveillance Centre, London, UK
g University of Siena, Siena, Italy
h Società Italiana di Medicina Generale (SIMG), Florence, Italy
i Hospital Clínico Universitario de Valladolid, Valladolid, Spain
j Hospital Clinic - University of Barcelona, Barcelona, Spain
k University of Padova, Padova, Italy
l Royal College of General Practitioners, British Global and Travel Health Association, London, UK

A R T I C L E   I N F O

Article history:
Received 28 January 2020
Received in revised form 1 June 2020
Accepted 7 June 2020
Available online 26 June 2020

Keywords:
Antigenic drift
Egg-adaptations
Influenza
Vaccine
Manufacturing

A B S T R A C T

Introduction: Influenza is associated with significant morbidity and mortality worldwide. Whilst vaccination is key for the prevention of influenza infection, there are many factors which may contribute to reduced vaccine effectiveness, including antigenic evolution via both antigenic drift and egg-adaptations. Due to the currently dissociated and indirect evidence supporting both the occurrence of these two phenomena in the egg-based manufacturing process and their effects on vaccine effectiveness, this topic remains a subject of debate.

Objective: To review the evidence and level of agreement in expert opinion supporting a mechanistic basis for reduced vaccine effectiveness due to egg-based manufacturing, using an expert consensus-based methodology and literature reviews.

Methods: Ten European influenza specialists were recruited to the expert panel. The overall research question was deconstructed into four component principles, which were examined in series using a novel, online, two-stage assessment of proportional group awareness and consensus. The first stage independently generated a list of supporting references for each component principle via literature searches and expert assessments. In the second stage, a summary of each reference was circulated amongst the experts, who rated their agreement that each reference supported the component principle on a 5-point Likert scale. Finally, the panel were asked if they agreed that, as a whole, the evidence supported a mechanistic basis for reduced vaccine effectiveness due to egg-based manufacturing.

Results: All component principles were reported to have a majority of strong or very strong supporting evidence (70–90%).

Conclusions: On reviewing the evidence for all component principles, experts unanimously agreed that there is a mechanistic basis for reduced vaccine effectiveness resulting from candidate influenza virus variation due to egg-based manufacturing, particularly in the influenza A/H3N2 strain. Experts pointed to surveillance, candidate vaccine virus selection and manufacturing stages involving eggs as the most likely to impact vaccine effectiveness.

⇑ Corresponding author.
E-mail addresses: Raja.Rajaram@seqirus.com (S. Rajaram), lejarazu@gmail.com (R. Ortiz de Lejarazu).

https://doi.org/10.1016/j.vaccine.2020.06.021
0264-410X/© 2020 The Author(s). Published by Elsevier Ltd.
This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
1. Introduction

Annually, influenza epidemics affect approximately 10–30% of the human population, with the European Centre for Disease Prevention and Control (ECDC) estimating that each year 4–50 million symptomatic cases of seasonal influenza occur in the European Union/European Economic Area; of these 4–50 million cases, around 70,000 are estimated to result in death [1]. Whilst the majority of influenza infections can be easily managed, infections can result in severe complications, particularly in at-risk groups such as pregnant women, young children, the elderly, and individuals with underlying conditions – such as human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS), chronic heart disease and/or chronic lung disease [2]. Amongst major influenza-related complications, secondary bacterial infections (particularly with Streptococcus pneumoniae) are especially linked with serious events, such as intensive care admission or death [3–5], and have been reported, in a 2008 study, to be associated with as much as 1 in 4 of all influenza-related fatalities [6]. The most effective method of combating influenza and averting any potentially severe complications is prevention with influenza vaccines [7,8].

1.1. Influenza vaccine effectiveness

Whilst influenza vaccines are an exceptionally useful tool, the effectiveness of current vaccines are suboptimal, with estimated effectiveness measures ranging from 40 to 60% when a vaccine is well matched with circulating strains; effectiveness is generally lower for the influenza A/H3N2 strain [9,10]. Alongside the mismatch resulting from egg-adaptations [11,12,14,18–20,23,29,32,35–38], there is currently limited direct evidence that egg-adaptation changes occurring during the egg-based manufacturing process negatively impact vaccine effectiveness, however, a potential link has been postulated in recent publications [29,44]. In the presence of the large body of evidence supporting the existence of antigenic drift, and the recent evidence for egg-adaptation induced virus changes, a link is likely. However, the current evidence base is limited and thus warrants investigation. The objective of this study is to determine whether there is a mechanistic basis for reduced vaccine effectiveness due to egg-based manufacturing.
2. Methods

A novel method to assess proportional group awareness and consensus – involving the generation and review of reference lists from a two-stage online survey eliciting expert opinion and a series of structured literature reviews – was employed between March and June 2018 (Fig. 1) [45]. To address the overarching study objective, it was deconstructed into the following component principles (CP): CP1) Presence of antigenic drift (virus variation), CP2) Stages in egg-based influenza vaccine manufacture and candidate virus vaccine selection, CP3) Egg-adaptation changes in influenza vaccine manufacturing process and CP4) Stage(s) in the manufacturing process most likely to impact influenza vaccine effectiveness (Fig. 2).

2.1. Structured literature review

The literature reviews adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and were carried out for each of the four research questions forming the subject of the expert survey [46]. The reviews were conducted by a single analyst and interrogated EMBASE (OVID) & MEDLINE via PubMed in March 2018. For all CPs, English-language research published within the past 30 years on human influenza viruses was included; speciality websites – such as the World Health Organisation (WHO), Centers for Disease Control and Prevention (CDC) and European Centre for Disease Prevention and Control (ECDC) – were also reviewed. For the research questions involving manufacturing, lower quality evidence – such as posters and conference materials – was also included. A full literature review-generated reference list was compiled for each CP, and the items contained within these lists were blinded to the panel. Full search criteria employed in this study can be found in Supplementary Table 2.

2.2. Assessment of expert opinion

Twelve European influenza experts were invited to form the expert panel; all individuals were recruited via professional networks. The inclusion criteria for the experts were: extensive experience and/or interests in influenza and influenza vaccination programmes, peer reviewed publications and memberships to learned organisations. A specialism in virology, public health, or general medical practice was also preferred.

Each component principle was examined by experts in series, using a two-stage online survey where each stage comprised two

Fig. 1. Insights protocol methodology.
rounds (Fig. 1). Each stage was methodologically the same and the number of survey rounds and concluding questions were pre-specified in the study protocol. The experts had an opportunity to have a one-on-one meeting with the research team to explain study methods and objectives before the survey started.

In the first survey stage (insight, or item generation phase), the expert panel were asked to provide their opinions on each CP, along with any supporting references. The responses and references were then coded by an analyst to form an expert opinion-generated reference list and, following removal of duplicates and application of the inclusion and exclusion criteria from the literature reviews, combined with the literature review-generated reference list.

In the second stage, a summary of each reference (or ‘item’) resulting from the literature was circulated amongst the experts. The summaries were prepared by the study analyst, a pharmacist with specialisation in health economics and systematic literature review experience, and comprised key text extracts from each reference, containing the answer to the research question and information on the methods of the study; this was performed regardless of whether the reference confirmed or contradicted the relevant component principle. Experts were randomly allocated into groups of two and were asked to assign a value on a 5-point Likert scale (1 = Not any evidence, 2 = Weak evidence, 3 = Moderate evidence, 4 = Strong evidence, 5 = Very strong evidence) to gauge the extent to which they agreed that each reference supported the CP; each group reviewed 20% of all identified references. No items could be removed from the list (all items were assessed) and consensus agreement from the experts that a reference supported a CP was achieved if the median agreement level on the Likert scale was $\geq 3.5$.

From all survey answers, a cumulative frequency of appearance and evidence rating for each item was recorded and analysed. Summary statistics were produced, with equal weightings given to each individual expert and the literature reviews. Results were anonymised and presented to the panel for discussion at a face-to-face meeting held in December 2018. A concluding consensus question was administered via an online survey sent shortly after the December meeting, wherein the panel were asked to answer yes or no when questioned if they agreed that, as a whole, the evidence reviewed supported a mechanistic basis for reduced vaccine effectiveness due to egg-based manufacturing.

3. Results

3.1. Participants

Of the 12 experts approached, 10 agreed to participate (83%). The final panel comprised three virologists, three public health physicians and four primary care physicians from four European countries (Italy, Spain, UK and Germany). All ten experts participated in all survey rounds. In total, the panel participated in five survey rounds and a concluding question.

3.2. Literature reviews

1256 publications were screened in the literature reviews and 170 progressed to assessment by the expert panel in the survey. The primary criteria for exclusion were non-human influenza virus(es) or the irrelevance of either topic or outcomes studied. The frequency of studies meeting inclusion criteria by CP were: antigenic drift 117/170 (69%), egg-adaptations 26/170 (15%), manufacturing steps 20/170 (12%) and virus variation referring specifically to egg-based manufacturing processes 7/170 (4%). For CPs involving questions on manufacturing (principles two and four), there was a greater number of professional websites – such as the WHO and CDC websites – and their publications considered by the experts as supportive evidence. The adjusted PRISMA chart with results of the literature reviews is presented below in Fig. 3, and full literature review search strategies can be found in Supplementary Table 2.

3.3. Survey of expert opinion

3.3.1. Survey round one and comparison with the literature reviews

The experts provided an average of 5.5, 4.4, 4.2 and 2.9 references for CPs one to four, respectively. Following removal of duplicate items, the combined total number of studies identified by the experts were 10, 9, 7 and 6 for CPs one to four, respectively. This was lower than the number of hits from the literature reviews, which were 117, 26, 20 and 7 respectively. However, both the literature reviews ($n = 149$) and the expert panel ($n = 11$) provided unique references (Fig. 4).
3.3.2. Survey round two

CPs one to four were rated as being supported by strong or very strong evidence in 95/123 (77%), 21/27 (78%), 19/21 (90%) and 7/10 (70%) of all references identified for each principle, respectively (Fig. 4 and Supplementary Table 1). Considering the overall body of evidence, references cited by both experts and the literature received the strongest levels of consensus and, with the exception of CP four (egg-adaptation changes in the manufacturing process), the experts scored evidence from the literature reviews as stronger than the evidence provided by fellow experts. Abbreviated lists of the highest ranked pieces of evidence for each CP can be found in Supplementary Tables 3–6.

3.4. Face-to-face meeting and concluding question

All ten panel members who agreed to participate in the survey attended the face-to-face meeting, where it was agreed that a common, uniform list of definitions for key terms would be desirable. All panellists also participated in the final survey delivered via an online survey and all ten agreed that there was a mechanistic basis of reduced vaccine effectiveness due to egg-based manufacturing processes. Experts also highlighted during this meeting that this phenomenon is likely to have a more pronounced effect on the influenza A/H3N2 strain compared with other influenza strains.

3.5. Summary of key findings

Anticipated patterns of evidence availability for component principles one to four (CP1 – CP4) were observed during this study. A large body of evidence was identified in support of CP1 (antigenic drift), with a stepwise decrease over the consecutive CPs, exploring manufacturing process of influenza vaccines (CP2), egg-adaptation changes (CP3), and the evidence observing egg-adaptation changes in the manufacturing process of the vaccines (CP4). Following the assessments of expert opinion, all component principles were reported to have a majority of strong, or very strong supporting evidence, with proportions of consensus ranging from 70% to 90% (Fig. 4, Supplementary Table 1). These key findings are further discussed below.

4. Discussion

This study examined the current evidence and expert opinion on virus variation mechanisms enabling escape from host immune systems and their role in egg-based influenza vaccine manufacturing on vaccine effectiveness. It was necessitated by the current absence of a formal consensus on the subject, despite the availability (albeit dissociated) of laboratory, genetic and surveillance data and driven by a demand to inform an understanding of the relative merits of competing manufacturing technologies in the field. The use of expert opinion in answering research questions under these circumstances is well established and recognised as having a legitimate level in the evidence hierarchy.

The approach adopted a stepwise interrogation of the four component principles comprising the research question using a novel proportional group awareness and consensus method.

The results shed light on the degree of differential awareness of evidence supporting each CP when compared to the corresponding literature review results. It is only to be expected that even the most well-read expert will not be able to generate a complete list of references answering a research question.

The literature review-generated reference lists were observed to complement the expert-generated reference lists with a higher number of both overall and unique items as expected (Supplementary Table 1). Furthermore, it was found that number of references were unique to the expert-generated reference list, enriching the overall evidence base for examining and demonstrating the value of the two parallel exercises. Explanations for this exclusivity included: references uploaded into the medical database with
missing abstract data, the reference date fell outside of time parameters of the study, and study-relevant keywords did not appear in the title and/or abstract. Of note, the evidence common to both the literature review-generated reference lists and the expert-generated reference lists achieved, on average, higher levels of agreement for their support of each CP. This indicates ‘reinforcement’, where experts were re-presented with items in round two that they themselves had suggested in round one. Items falling outside this common list may have achieved a lower level of comparative consensus, potentially mediated through limited expert awareness, and a consequent inability to confidently agree the level of support. Furthermore, the method highlighted the equally important phenomenon of ‘prompting’ where items unique to the literature review-generated reference lists reminded the experts of key evidence in support of the CPs, which they then agreed with when presented.

For each component principle, relevant references were reviewed (see appendices). Within each component principle, influential studies were identified. Though the overarching statement remained unsupported in its entirety as expected, the following studies reflect the highest level of the support for each component of the statement from the experts’ perspective. A summary of these, compelling, references are discussed in next paragraphs.

The reviewed evidence for the antigenic drift of influenza virus (CP1), describes it as an established and accepted phenomenon. It comprised of mostly of genetic, antigenic, or phylogenetic characterization of influenza virus. Smith et al. 2004 [47], both the highest scored and relatively most cited publication in the literature on this topic, analysed antigenic data from 273 viral isolates of 35 years of influenza surveillance. In addition to observance of the antigenic drift, the authors calculated yearly rate of the phenomenon occurring, as well as the rate of amino acid substitutions. Similar level of scrutiny was reported in the publication by Bedford at al. 2014 [48], who combined genetic characterization with antigenic maps in their approach and estimated the rate of antigenic drift of four lineages of influenza virus, showing that A/H3N2 evolved faster and in a more punctuated fashion than influenza A/H1N1, Influenza B/Victoria, and B/Yamagata respectively. Over a hundred other reviewed publications in support of the CP1 of
the antigenic drift, agree to the existence of the phenomenon, with discussions mainly focused around: 1) seasonal presence and divergence from reference vaccine strains [49–73], 2) drivers of influenza epidemics [51–54,61,74–77], 3) in-depth understanding of the phenomenon [62,72,76,78–89], and 4) its impact on influenza vaccination [58,82,90–96].

The second component principle was incorporated in the study to ensure equal awareness of manufacturing steps among experts. As a less scientific, but more of an industrial subject, the evidence published in the literature was more limited. The reviewed literature was presented to the experts in form of links to full publications in this case, as the sought information was usually scattered along the text or/and embedded in figures. The experts highlighted evidence from Minor et al. 2010 [97], examining challenges of production of influenza vaccines, as the most informative of the reviewed articles. Evidence from CDC and WHO websites and books were also highly rated.

The identified and reviewed evidence for the CP3 on the egg-adaptations phenomenon, comprised mostly of antigenic and genetic characterization studies. Zost et al. 2017 [44] was the highest rated evidence in component principle 3. It is interesting to note that, four the experts cited Robertson et al. 1987 [21], as their suggested evidence. Robertson et al. 1987 [21] was outside of the time frame set in the literature review, suggesting possible relaxation of the inclusion criteria in the future updates of this subject. In addition, it was observed that several articles have been published after the literature review reported here was conducted, these newly published articles are not described here.

Similarly to antigenic drift (CP1), the subject of egg-adaptations is being investigated in the reviewed literature for 1) Most of the reviewed publications on CP3 aimed for better understanding of the process and to identify the substitutions associated with egg-adaptations in particular [17,19,21,24,29,30,33,44,98–100] and 2) implications on the influenza vaccine effectiveness [17,19,20,32,44,101].

The CP4 was aiming to draw from the two preceding component principles, by answering the question of which steps in egg-based manufacturing are most likely to be responsible for reduction in vaccine effectiveness. The reviewed evidence directly, or indirectly pointed to steps of manufacturing involving eggs, as the possible culprit of the vaccine ineffectiveness, with Raymond et al. 2016 [17] and et al. 2017 [44] as highest rated supporting evidence. Several authors highlighted that, despite mutations occurring due to propagation in eggs, the effect on antigenicity and vaccine effectiveness is difficult to predict and remains a subject of a debate Petrie et al. 2018 [50].

A strength of the group awareness and consensus approach used in this study over similar methods – such as the Delphi consensus methodology – is that all evidence was assessed by the experts, regardless of whether it supported or contradicted the relevant component principle, minimising the risk of selection bias. Furthermore, there was no attrition of experts, with all those participating in the first round of the questionnaire participating in the second, this is often a weakness of similar approaches.

There appear to be two major factors that can impact influenza vaccine effectiveness – the characteristics of the subject being vaccinated and the vaccine match to the circulating strain [10]. Whilst the former cannot be controlled, the latter can – to a degree – be managed by providing a good match between the CVV used to produce the vaccine seeds and the virus in the end-product (i.e. the vaccine). As the evidence shows, vaccines that are well-matched generally offer better protection against influenza and, therefore, a good match can be a predictor of good vaccine effectiveness.

A key finding from this study is that, vaccine production technologies not involving eggs – such as cell-based or recombinant flu vaccine manufacturing – could avoid shortcomings associated with egg-adaptation changes, resulting in improved effectiveness of influenza vaccines [11,56]. This study also found that, barring surveillance and CVV selection, manufacturing steps involving eggs were most likely to cause virus mismatch and decreased vaccine effectiveness. Although this outcome seems plausible based on the current evidence, to our knowledge this is the first study strongly linking these phenomena with egg-based manufacturing processes.

This may have implications for patients, prescribers, and payors, as an important factor to consider when choosing an influenza vaccine. Furthermore, during the face-to-face meeting, the complexity of the CPs became apparent, particularly with regards to working definitions. This also highlighted the importance of expert consensus on this subject, alongside identifying potential areas for ongoing educational activities and further expert collaboration.

5. Limitations

A potential limitation of this study was the number of experts recruited to the panel. This potential limitation could be ameliorated by distributing the second-round survey to a larger group of experts, and potentially relaxing the inclusion criteria for this round to include those with a working understanding of the therapy area. This may also help to identify areas for continuing educational activities. Importantly, the method employed has previously reported on proportional group awareness and consensus with a minimum number of three experts, also contributing to an amelioration of this limitation [45]. Secondly, a potential source of bias in this study may have been the distribution of the reference lists over five pairs of experts, meaning 20% of any reference list was reviewed by only two of the ten experts. This approach was adopted to enable the assessment of the large body of evidence by the experts in a timely manner. However, all participants were experts in this field, and therefore were assumed to have a similar level of knowledge and understanding of the topics presented, allowing them to provide accurate and representative assessments of the evidence.

The issue of egg adaptations has come to the forefront of many vaccine discussions over the last two years, however as the lock date for the literature review component of this study was mid-2018, many of the recently published literature on this topic were not assessed. The implication of this is that, newer studies comparing the efficacies of cell-based and egg-based influenza vaccines, alongside studies exploring avenues such as the prevention or mitigation of egg-adaptation changes, have unfortunately not been included in this study [102–105]. It would be of potential interest to perform an update of this project in the coming years to determine the impact that new knowledge of egg-adaptations has had on vaccination.

Furthermore, whilst experts were asked whether they agreed that there is a mechanistic basis for reduced vaccine effectiveness due to egg-based manufacturing, they were not asked to provide any information or opinions on the frequency of occurrence or severity of egg-adaptation changes. Indeed, whilst there is support for the mechanistic basis of these changes, currently there is limited evidence available to inform how frequently these changes occur nor how significant an effect they have on vaccine effectiveness. Further research will be required to determine this important information.

6. Conclusions

The existence of antigenic drift and egg-adaptation changes is well documented and has been demonstrated in numerous laboratory studies. However, the extent to which these are attributable to
the egg-based manufacturing of influenza vaccines, and the impact of the phenomena on vaccine effectiveness, remains unknown. In the present study, a group of European experts in influenza unanimously concluded that there is a mechanistic basis for reduced vaccine effectiveness resulting from candidate influenza virus variation due to egg-based manufacturing.

Overall, evidence from this study suggests that, despite being an established way of producing influenza vaccines worldwide, egg-based manufacturing likely results in reduced influenza vaccine effectiveness due to virus egg-adaptation changes. This effect was believed to be particularly pronounced against certain strains such as influenza A/H3N2. Whilst more work is needed in this area to determine the exact contribution of these changes to vaccine effectiveness, this phenomenon should be a key consideration in global public health infrastructure planning.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Acknowledgements**

Dr Alexander T. Hardy of Medialis Ltd provided Medical Writing support for this manuscript. Dr P.S. (German general practitioner expert), participated in the research survey, but was not involved in the manuscript writing.

**Potential Conflict of Interest**

This work was funded in whole by Seqirus, a pharmaceutical company who manufacture flu vaccines. All external key opinion leaders were remunerated for their time in line with the fair market value policy of Seqirus.

**Appendix A. Supplementary material**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2020.06.021.

**References**

[1] European Centre for Disease Prevention and Control. Disease facts about seasonal influenza. Eur Cent Dis Prev Control; n.d. https://www.ecdc.europa.eu/en/seasonal-influenza/facts (accessed January 15, 2020).

[2] World Health Organisation. Vaccines against influenza WHO position paper - November 2012 2012;47:461–76.

[3] Machtyre CR, Chughhai AA, Barnes M, Ridda I, Seale H, Toms R, et al. The role of pneumonia and secondary bacterial infection in fatal and serious outcomes of pandemic influenza A(H1N1)pdm09. BMC Infect Dis 2018;18:637. https://doi.org/10.1186/s12879-018-3548-0.

[4] Morris DE, Cleary DW, Clarke SC. Secondary bacterial infections associated with influenza pandemics. Front Microbiol 2017;8:1041. https://doi.org/10.3389/fmicb.2017.01041.

[5] Metzelerik ML, Masterton RG, Lode H, File TM, Babincak T. Epidemiology, microbiology, and treatment considerations for bacterial pneumonia complicating influenza. Int J Infect Dis 2012;16:e321–31. https://doi.org/10.1016/j.ijid.2012.01.003.

[6] Gupta RK, George R, Nguyen-Van-Tam JS. Bacterial pneumonia and pandemic influenza planning. Emerg Infect Dis 2008;14:1187–92. https://doi.org/10.3201/eid1408.070592.

[7] Shaw MW, Xu X, Li Y, Normand S, Ueki RT, Kinumoto GY, et al. Reappearance and global spread of variants of influenza B/Victoria/2/87like lineages in the 2000–2001 and 2001–2002 seasons. Virology 2002;303:1–8. https://doi.org/10.1006/viro.2002.1710.

[8] Mameli C, Cocchi E, Funagalli M, Zuccotti G. Influenza vaccination: effectiveness, indications, and limits in the pediatric population. Front Pediatr 2019;7:317. https://doi.org/10.3389/fped.2019.00317.

[9] Vaccine Effectiveness: How Well Do the Flu Vaccines Work? | CDC 2019. https://www.cdc.gov flu/vaccines-work/vaccineeffect.htm (accessed April 23, 2020).

[10] Influenza vaccine effectiveness. Eur Cent Dis Prev Control; 2019. http://ecdc.europa.eu/en/seasonal-influenza/prevention-and-control/vaccine-effectiveness (accessed April 30, 2019).

[11] Vaccine Effectiveness: How Well Do the Flu Vaccines Work? | CDC 2019. https://www.cdc.gov flu/vaccines-work/vaccineeffect.htm (accessed April 27, 2019).

[12] Oxford JS, Newman R, Corcoran T, Bootman J, Major D, Yates P, et al. Direct isolation in eggs of influenza A (H1N1) and B viruses with haemagglutinins of different antigenic and amino acid composition. J Gen Virol 1991;72:185–9.

[13] Parker L, Wharton SA, Martin SR, Gross K, Lin Y, Liu Y, et al. Effects of egg-adaptation on receptor-binding and antigenic properties of recent influenza A (H3N2) vaccine viruses. J Gen Virol 2016;97:333–44. https://doi.org/10.1099/jgv.0.00457.

[14] Kishida D, Fujiyaki S, Yokoyama M, Sato H, Saito R, Bremmatu H, et al. Evaluation of influenza virus A/H3N2 and B vaccines on the basis of cross-reactivity of postvaccination serum human antibodies against influenza viruses A/H3N2 and B isolated in MDCK cells and embryonated hen eggs. Clin Vaccine Immunol 2011;18:897–908. https://doi.org/10.1128/CVI.01725-11.

[15] Nicolson C, Harvey R, Engelhardt OG, Robertson JS. The ability of a non-egg adapted (cell-like) A(H1N1)pdm09 virus to egg-adapt at HA loci other than 222 and 223 and its effect on the yield of viral protein. PLoS ONE 2016;11. https://doi.org/10.1371/journal.pone.0166761.

[16] Robertson JS, Nicolson C, Major D, Robertson EW, Wood JM. The role of amniotic passage in the egg-adaptation of human influenza virus is revealed by haemagglutinin sequence analysis. J Gen Virol 1993;74:2407–51. https://doi.org/10.1099/jgv.0.09862.

[17] Raymond DD, Stewart SM, Lee J, Ferdman J, Bajic G, Do KT, et al. Influenza immunization elicits antibodies specific for an egg-adapted vaccine strain. Nat Med 2016;22:1465–9. https://doi.org/10.1038/nm.4145.

[18] Vooladhari S, Justwicz DM, Gubareva LV, Webster RG. Selection of a single amino acid substitution in the haemagglutinin molecule by chicken eggs can render influenza A virus (H3) candidate vaccine ineffective. J Virol 1995;69:4888–97.

[19] Katz JM, Webster RG. Efficacy of inactivated influenza A (H2N2) vaccines grown in mammalian cells or embryonated eggs. J Infect Dis 1989;160:191–8. https://doi.org/10.1093/infdis/160.1.191.

[20] Robertson JS, Cook P, Nicolson C, Newman R, Wood JM. Mixed populations in influenza vaccine virus strains. Vaccine 1994;12:1317–22. https://doi.org/10.1016/0264-410x(94)90058-8.

[21] Robertson JS, Bootman JS, Newman R, Oxford JS, Daniels RS, Webster RG, et al. Structural changes in the haemagglutinin which accompany egg adaptation of the influenza A/H3N2 virus. Virology 1987;160:31–7. https://doi.org/10.1016/0042-6822(87)90040-7.

[22] Gubareva LV, Wood JM, Meyer WJ, Katz JM, Robertson JS, Major D, et al. Codominant mixtures of viruses in reference strains of influenza virus due to host cell variation. Virology 1994;199:89–97. https://doi.org/10.1006/viro.1994.1100.

[23] Wood JM, Oxford JS, Dunleavy U, Newman RW, Major D, Robertson JS. Influenza A (H1N1) vaccine efficacy in animal models is influenced by two amino acid substitutions in the haemagglutinin molecule. Virology 1989;171:214–21. https://doi.org/10.1016/0042-6822(89)90058-5.

[24] Rocha EP, Xu X, Hall HE, Allen JR, Regnery HL, Cox NJ. Comparison of 10 influenza A (H1N1 and H3N2) haemagglutinin sequences obtained directly from clinical specimens to those of MDCK cell- and egg-grown viruses. J Gen Virol 1993;74(Pt 11):2513–8. https://doi.org/10.1099/jgv.0.1971-74.11-2513.

[25] McLean KA, Goldin S, Nannei C, Sparrow E, Torelli G. The 2015 global influenza planning. Emerg Infect Dis 2008;14:1187–92. https://doi.org/10.3201/eid1408.070592.

[26] Harding AT, Heaton NS. Efforts to improve the seasonal influenza vaccine. Vaccines 2018;6. https://doi.org/10.3390/vaccines6020019.

[27] How Influenza (Flu) Vaccines Are Made | CDC 2019. https://www.cdc.gov/flu/ prevent/how-flu-vaccine-made.htm (accessed April 23, 2020).

[28] Ping J, Lopes TJS, Nidom CA, Ghedin E, Macken CA, Fitch A, et al. Development of high-yield influenza A virus vaccines. Nat Commun 2015;6:8148. https://doi.org/10.1038/ncomms9148.

[29] Wu NC, Zost SJ, Thompson AJ, Oyen D, Nycholat CM, McBride R, et al. Structural explanation for the low effectiveness of the seasonal influenza H3N2 vaccine. PLoS Pathog 2017;13. https://doi.org/10.1371/ journal.ppat.1006682.

[30] Skowronska DM, Janusz NZ, De Serres G, Sabaudec S, Eshaghia A, Dickinson JA, et al. Low 2012–13 influenza vaccine effectiveness associated with mutation in the egg-adapted H3N2 vaccine strain not antigenic drift in circulating viruses. PLoS ONE 2014;9. https://doi.org/10.1371/journal.pone.0092153.
[80] Stray SJ, Pittman LB. Subtype- and antigenic site-specific differences in biophysical influences on evolution of influenza virus hemagglutinin. Virol J 2012;9:91. https://doi.org/10.1186/1743-422X-9-91.

[81] Rambaut A, Pybus OC, Nelson M, Viboud C, Taubenberger JK, Holmes EC. The genomic and epidemiological dynamics of human influenza A virus. Nature 2008;453:615–9. https://doi.org/10.1038/nature06934.

[82] Retanaal M, Abed Y, Rheaume C, Baz M, Boivin G. In vitro and in vivo evidence of a potential A(H1N1)pdm09 antigenic drift mediated by escape mutations in the haemagglutinin Sa antigenic site. J Gen Virol 2017;98:1224–31. https://doi.org/10.1099/jgv.0.008900.

[83] Shih AC, Hsiao TC, Ho MS, Li WH, AC-C S, T-C H, et al. Simultaneous amino acid substitutions at antigenic sites drive influenza A hemagglutinin evolution. Proc Natl Acad Sci U S A 2007;104:6283–8. https://doi.org/10.1073/pnas.0701396104.

[84] Westgeest KB, Bestebroer TM, Sproonen MJL, Gao J, Couzens L, Osterhaus ADME, et al. Optimization of an enzyme-linked lectin assay suitable for rapid antigenic characterization of the neuraminidase of human influenza A(H3N2) viruses. J Virol Methods 2015;217:55–63. https://doi.org/10.1016/j.viromet.2015.02.014.

[85] Abed Y, Hardy I, Li Y, Boivin G. Divergent evolution of hemagglutinin and neuraminidase genes in recent influenza A(H3N2) viruses isolated in Canada. J Med Virol 2002;67:589–95. https://doi.org/10.1002/jmv.10143.

[86] Al Faress S, Cartet G, Ferraris O, Norder H, Valette M, Liou D, et al. Divergent genetic evolution of hemagglutinin in influenza A H1N1 and A H1N2 subtypes isolated in the south-France since the winter of 2001–2002. J Clin Virol Off Publ Pan Am Soc Clin Virol 2005;33:230–6. https://doi.org/10.1016/j.jcv.2004.11.016.

[87] Inoue E, Iketi M, Takahashi N, Osawa Y, Okazaki K. Phylogenetic analyses of pandemic influenza A (H1N1) virus in university students at Tobetsu, Hokkaido, Japan. Microbiol Immunol 2012;56:273–9. https://doi.org/10.1016/j.mib.2012.04.022.

[88] Kilbourne ED, Johansson BE, Gajower B, EDK, BE J. Diverse and disparate evolution in nature of influenza A virus hemagglutinin and neuraminidase glycoproteins. Proc Natl Acad Sci U S A 1990;87:786–90.

[89] Skowronski DM, Masaro C, Kwindi TL, Mak A, Petric M, Li Y, et al. Estimating seasonal influenza vaccine vaccine effectiveness against laboratory-confirmed influenza using a sentinel physician network: results from the 2005–2006 season of dual A and B vaccine mismatch in Canada. Vaccine 2007;25:2842–51. https://doi.org/10.1016/j.vaccine.2006.10.002.

[90] Chambers BS, Parkhouse K, Ross TM, Alby K, Hensley SE. Identification of hemagglutinin residues responsible for H1N2 antigenic drift during the 2014–2015 influenza season. Cell Rep 2015;12:1–6. https://doi.org/10.1016/j.celrep.2015.05.005.

[91] Belavoin SS, Bychilov D, Benner C, Ripatti S, Ojala T, Kankainen M, et al. Genome-wide analysis of evolutionary markers of human influenza A(H1N1) pdm09 and A(H3N2) viruses may guide selection of vaccine strain candidates. Genome Biol Evol 2015;7:3472–83. https://doi.org/10.1093/gbe/evv240.

[92] Skowronski DM, Chambers C, Sabadics S, De Serres C, Winter A-L, Dickinson JA, et al. A perfect storm: impact of genomic variation and serial vaccination on low vaccine influenza effectiveness during the 2014–2015 season. Clin Infect Dis Off Publ Infect Dis Soc Am 2016;63:21–32. https://doi.org/10.1093/cid/ciw176.

[93] Pebody R, Warburton F, Ellis J, Andrews N, Potts A, Cottrell S, et al. Effectiveness of seasonal influenza vaccine for adults and children in preventing laboratory-confirmed influenza in primary care in the United Kingdom: 2015/16 end-of-season results. Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull 2016;21. https://doi.org/10.2807/1560-7917.ES.2016.21.38.30349.

[94] Abed Y, Hardy I, Li Y, Boivin G. Divergent evolution of hemagglutinin and neuraminidase glycoproteins. Proc Natl Acad Sci U S A 1990;87:786–90.

[95] Lugovtsev VY, Vodeiko GM, Strupczewski CM, Ye Z, Levandowski RA. Generation of the influenza B viruses with improved growth phenotype by substitution of specific amino acids of hemagglutinin. Virology 2007;365:315–23. https://doi.org/10.1016/j.virol.2007.04.006.

[96] Stevens J, Chen LM, Carney PJ, Garten R, Foust A, Le J, et al. Receptor specificity of influenza A H3N2 viruses isolated in mammalian cells and embryonated chicken eggs. J Virol 2010;84:8287–9. https://doi.org/10.1128/JVI.00558-10.

[97] Widjaja I, Ryushina N, Webster RG, Webby RJ. Molecular changes associated with adaptation of human influenza A virus in embryonated chicken eggs. Virology 2006;350:137–45. https://doi.org/10.1016/j.virol.2006.02.020.

[98] Newman RW, Jennings R, Major DL, Robertson JS, Jenkins R, Potter CW, et al. Immune response of human volunteers and animals to vaccination with egg-grown influenza A (H1N1) virus is influenced by three amino acid substitutions in the haemagglutinin molecule. Vaccine 1993;11:400–6. https://doi.org/10.1016/0264-410x(93)90027-9.

[99] Barr IG, Donis RO, Katz JM, McCauley JW, Ogatigi T, Trusheim H, et al. Cell culture-derived influenza vaccines in the severe 2017–2018 epidemic season: a step towards improved influenza vaccine effectiveness. npj Vaccines 2018;3. https://doi.org/10.1038/s41541-018-0079-x.

[100] Izuijita HS, Chilliard Y, Kelman J, Wei Y, Lu Y, Xu W, et al. Relative effectiveness of cell-cultured and egg-based influenza vaccines among elderly persons in the United States, 2017–2018. J Infect Dis 2019;220:1255–64. https://doi.org/10.1093/infdis/jiy716.

[101] Wu NC, Lv H, Thompson AJ, Wu DC, Ng WWS, Kadam RJ, et al. Preventing an antigenically disruptive mutation in egg-based H3N2 seasonal influenza vaccines by mutational incompatibility. Cell Host Microbe 2019;25(8):836–844. https://doi.org/10.1016/j.chom.2019.04.013.

[102] Boikos C, Sylvester GC, Sampalis JS, Mansi JA. Relative effectiveness of the cell-cultured quadrivalent influenza vaccine compared to standard, egg-derived quadrivalent influenza vaccines in preventing influenza-like illness in 2017–2018. Clin Infect Dis n.d. https://doi.org/10.1093/cid/ci261.371.