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Javier Álvarez Aldeán[1]
Ignacio Salamanca[2]
Daniel Ocaña[3]
José Luis Barranco[4]
Stefan Walter[5]

Effectiveness of cell culture-based influenza vaccines compared with egg-based vaccines: What does the literature say?

[1]Pediatrics Department. Hospital Costa del Sol, Málaga, Spain
[2]Studies and Research Unit, Instituto Hispalense de Pediatría, Seville, Spain
[3]Health Center, Algeciras-Norte, Cadiz, Spain
[4]Medicina Preventiva. Hospital Reina Sofía, Córdoba, Spain
[5]Department of Medical Specialties and Public Health, Emerging Research in Epidemiology and Diagnosis of Infectious Diseases, Universidad Rey Juan Carlos, Madrid, Spain

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ABSTRACT

Introduction. Influenza vaccination is an effective way of reducing the burden of seasonal influenza. Chicken egg embryos are the most common source of influenza vaccines, but cell culture production has emerged as an alternative that could be advantageous. This article reviews the available literature on the efficacy/effectiveness of cell culture-based influenza vaccines compared with egg-based vaccines.

Methods. We conducted a review of the actual literature and analyzed those studies comparing the effectiveness of cell culture-based and egg-based vaccines in the last ten years.

Results. Eight studies were analyzed; 1 was a clinical trial and 7 were retrospective cohort studies. The clinical trial found no significant differences in the efficacy of both vaccines with respect to placebo. The results of the observational studies were inconsistent and relative effectiveness varied among studies, even though most were performed during the same season, and in some cases, in the same region and using the same data records. Furthermore, in most studies, the comparisons between vaccines were not statistically significant.

Conclusions. There is insufficient evidence that cell culture-based vaccines are superior to egg-based vaccines in terms of efficacy/effectiveness.

Keywords: Influenza, influenza virus, egg-based vaccine, cell culture-based vaccine.

Correspondence
Javier Álvarez Aldeán
Head of Pediatrics. Hospital Costa del Sol
A-7, Km 187, 29603 Marbella, Málaga, Spain
E-mail: javieraldean@icloud.com

Efectividad de la vacuna antigripal generada en cultivo celular frente a la producida en huevo: ¿Qué dice la literatura actual?

ABSTRACT

Introducción. La vacunación frente a la gripe es el método más efectivo para reducir el impacto de la gripe estacional. Los embriones de huevo de gallina son el método más común de fabricación de vacunas antigripales, pero la propagación en cultivos celulares ha emergido como una alternativa que podría ofrecer alguna ventaja. El objetivo de este artículo es hacer una revisión de la literatura disponible sobre la efectividad de vacuna antigripal generada en cultivos celulares frente a la vacuna producida en huevo.

Métodos. Se realizó una búsqueda bibliográfica de los estudios comparativos entre la vacuna propagada en cultivos celulares y la producida en huevo con respecto a su efectividad publicados en los últimos diez años.

Resultados. De los siete estudios analizados, uno fue un ensayo clínico y seis fueron estudios de cohortes retrospectivos. Los resultados del ensayo clínico mostraron que no existían diferencias significativas en cuanto a la eficacia de ambas vacunas. Con respecto a los estudios observacionales, los resultados fueron poco consistentes, con efectividades relativas que fueron muy diferentes entre estudios a pesar de que la mayoría se realizaron durante la misma temporada, y en algunos estudios, en la misma región y utilizando el mismo registro de datos. Además, en la mayoría de los estudios no hubo significación estadística.

Conclusiones. No existen evidencias suficientes de que la vacuna producida en cultivo celular sea superior a la generada en huevo con respecto a su efectividad.

Palabras clave: Influenza, virus gripe, cultivos celulares, huevo, vacunas

INTRODUCTION

The influenza virus is one of the most serious human pathogens and one of the leading causes of acute respiratory infections. Seasonal influenza causes between 4 and 50 million symptomatic cases in the European Union each year, affecting about 10%-30% of the population and causing the deaths of between 15,000 and 70,000 people annually [1].

Vaccination is an effective method of preventing influenza infections and associated complications [2]. Influenza vaccines consist of 3 (trivalent) or 4 (quadrivalent) strains of influenza virus (A/H1N1, A/H3N2, B/Yamagata, B/Victoria). In February of each year, the World Health Organization (WHO) determines the annual composition of influenza vaccines for the following season in the northern hemisphere based on global epidemiological data of virus strains circulating in the previous period. The viral formulation is then distributed to vaccine manufacturers who produce the viruses, generating enough doses for the population.

Currently, influenza vaccines are mainly produced in fertilized chicken eggs, and the efficacy and safety of this system is well established. However, egg-based manufacturing also has some disadvantages, such as limited production capacity, prolonged production time, and the possibility of allergic reactions caused by egg-derived proteins. The isolation and propagation of the virus in eggs can also cause mutations in the amino acids around the binding site of the hemagglutinin (HA) receptor that could affect their effectiveness [3,4].

Novel alternatives to the manufacture of the influenza vaccine in eggs have been in development for some time [5]. The 2 formulas most commonly used in production worldwide are the generation of recombinant vaccines and propagation in cell lines [6-8]. Since the end of 2019, a cell-based influenza vaccine has been marketed in Spain [9]. This is a quadrivalent vaccine produced in mammalian cells (Madin-Darby Canine Kidney, MDCK) that have been shown to be suitable for the propagation of the virus [10]. This methodology has a number of advantages in the propagation process. For a start, it does not appear to make significant changes in the HA amino acid sequence [11]. Moreover, cell lines are widely characterized and can be stored for future use without repeating a full-range test. Because some viruses grow better in cells, the time required for generating large-scale rearrangements is reduced and scalability is improved. However, this system also has some limitations, cells must be free of adventitious viruses, which requires extensive screening of cell lines [12]. Furthermore, the implementation of this system would involve the creation of new production facilities [12].

In recent years, various studies have analyzed how cell culture-based influenza vaccines function, particularly in terms of tolerability and immunogenicity [6,13-15]. However, to date no clinical trials have directly compared the efficacy/effectiveness of cell-propagated influenza vaccines with vaccines generated in chicken eggs. A 3-arm randomized clinical trial that studied vaccines produced in embryonic hen eggs, cell culture-generated vaccines, and placebo has been published, but the vaccines were not compared directly against placebo. The data available to date are based mainly on observational single-season studies which are difficult to compare due to methodological differences (case definition, virological confirmation) and the high variability of the influenza virus in each season.

In an attempt to determine the comparative efficacy/effectiveness of cell culture-based and egg-based influenza vaccines, our group of experts in epidemiology, pediatrics, family medicine, and scientific methodology analyzed the evidence published in the last 10 years. This article presents our results and conclusions.

METHODS

We conducted a literature search of the available scientific studies comparing the efficacy/effectiveness of cell culture-based and egg-based influenza vaccines in MEDLINE/PubMed and Scopus between November 2010 and November 2020, restricted to articles published in English or Spanish. In total, 140 articles were retrieved. After excluding publications with the format of reviews, comments on articles, editorials and opinion articles, 8 publications were finally included in the analysis: 1 clinical trial and 7 observational studies. The authors met via teleconference and discussed about the selected bibliography.

RESULTS

Analysis of studies comparing egg-based influenza vaccines and cell culture-based vaccines. Table 1 summarizes the characteristics of the 7 observational studies in which the effectiveness of cell-based influenza vaccine was compared with chicken egg-based influenza vaccine. The efficacy of both vaccines with respect to placebo was analyzed in only 1 study [16]. This study, published in 2010, also assessed the tolerability and immunogenicity of the vaccines using a randomized, observer-blind, placebo-controlled design in individuals aged 18 to 49 years. Adults from the US, Finland, and Poland were included during the 2007-2008 season. Participants were randomized to receive (1: 1: 1) trivalent cell-cultured vaccine, trivalent egg-based vaccine, and placebo. Influenza was defined as influenza-like illness (ILI) with a temperature of ≥ 37.8ºC plus sore throat/cough and confirmation by PCR. The results of the study that included 11,404 randomized subjects (3,776 received cell culture-derived vaccine, 3,638 egg-derived vaccine, and 3,843 placebo) showed that both vaccines were effective and differences were not statistically significant. Overall efficacy for all strains compared to placebo was 69.5% for cell culture-derived vaccine and 63.0% for egg-based vaccine [16]. The two vaccines also showed high immunogenicity and were well tolerated [16]. It is important to note that, since this was not a head-to-head clinical trial of the two types of vaccines but instead a comparison against placebo, the results lacked sufficient statistical power to make a valid direct com-

parison the trial. Another limitation is that this was an observer-blind trial which may have led to multiple biases. Nor were details provided on whether the cell culture-based influenza vaccine had previously been adapted in egg, a process that was common in the early years of cell-based vaccine production [4]. The efficacy of the vaccines was analyzed during 1 season only, despite the well-known ability of the influenza virus to undergo significant antigenic variations every year [17].

In the last 10 years, 6 observational studies have evaluated the effectiveness of the influenza vaccine produced in fertilized hen eggs compared to cell culture-derived vaccines. Most were conducted in the 2017-2018 season, which saw a high incidence of influenza cases. DeMarcus et al. [18] performed a study using a negative test design that included PCR-confirmed ILI cases that were treated at U.S. Department of Defense medical centers between October 1, 2017, and April 28, 2018. The study included 4,037 samples from 1,757 cases (43.5 %) and 2,280 controls. Thirty percent (531) of the cases had been vaccinated, 11% with cell culture-derived vaccines and 19% with egg-derived vaccines. Subjects of all ages (from 6 months to over 65 years), with a mean age of 24 years, were included. Statistically significant differences in favor of the egg-based vaccine were only found for H1N1 strains, which showed efficacy rates of 86% versus 61% for all age groups and 88% versus 56% in children (Table 1). The analysis of all strains in all age groups showed a higher, but not statistically significant, relative vaccine effectiveness (VE) for the egg-derived vaccine (VE 53 % vs. 46%) [18]. An evaluation of the methodological aspects of the article reveals a significant difference in the age distribution of patients who received each type of vaccine: most of the children who were vaccinated received the egg-derived vaccine (79.0%)[18]. Thus, since the confidence intervals will be smaller in this group, it is easier to achieve statistically significant effectiveness. Furthermore, comorbidities were not taken into account during the study. Another possible bias is that the study was conducted in members of the U.S. Department of Defense, a population that generally has better health than the real-world population in whom the flu vaccine is usually targeted. The timing of vaccination may also have had an effect, since the chicken egg-derived vaccine was administered before the cell culture-derived vaccine. This may be of great importance, since the effectiveness of the influenza vaccine is estimated to fall by 6%-12% each month [19], thus favoring greater VE in the cell culture-based vaccine. The study has other limitations, such as a small sample size, a restrictive subanalysis, and follow-up for a single season.

Another study conducted in the U.S. in the 2017-2018 season included hospitalized subjects aged ≥ 4 years enrolled in the California Kaiser Permanente registry between October 1, 2017 and May 31, 2018 [20]. The effectiveness of the influenza vaccine against hospitalization for laboratory-confirmed influenza was assessed in 5,239 individuals who received the egg-based vaccine, 232 people who received the cell culture-based vaccine, and 2,661 people who were not vaccinated. No differentiation was made between trivalent or quadrivalent vaccines. After analyzing the results, a statistically significantly greater relative VE was observed with the egg-based vaccine in patients < 65 years of age for the A/H3N2 strain only, with negative effectiveness (-44%; 95% CI : -99, -4) [20]. However, since it was an inactivated vaccine and cannot cause the disease, this outcome has no clinical implications. The study has little statistical power due to its small sample size. Furthermore, it has multiple biases, such as the exclusion from the analysis of patients without confirmed influenza and the fact that vaccinated patients had more comorbidities. The interval between the onset of symptoms and the influenza test was not reported. Moreover, a remarkably smaller number of patients received the cell culture-based vaccine, revealing an uneven distribution of vaccines. Overall, the results of this article are inconsistent.

Klein *et al.* [21] also used the Californian Kaiser Permanente patient registry during the 2017-2018 season. In this study, the relative VE of the quadrivalent cell-based influenza vaccine was compared to the egg-based vaccine in standard, trivalent, and quadrivalent doses, in patients aged 4 to 64 years. Although the study did not show statistically significant differences with respect to relative VE, the egg-based vaccine showed superiority in patients younger than 65 years (Table 1). Again, however, the study found a significant imbalance in the distribution of vaccines in the children's group. While 50% of individuals who received the cell culture-based vaccine were children between the ages of 4 and 17, only 23% of the individuals who received the egg-based vaccine belonged to this age group.

The study conducted by Boikos *et al.* [22] estimated the relative VE of the cell-cultured quadrivalent vaccine compared to the egg-derived vaccine in the US during the 2017-2018 season, using electronic medical records from primary care practices with a catchment population of over 55 million people. All patients ≥ 4 years who presented with ILI without microbiological confirmation of influenza, who were seen at any of the centers included in the database, and who had been vaccinated at least 14 days before the onset of symptoms were included in the study. A multivariate logistic regression was performed adjusting for possible confounding factors defined *a priori*. Sensitivity analysis was also performed using propensity score matching and analysis by age subgroups. A total of 1,353,862 subjects were included, 93% of whom had received the egg-based vaccine and 7% the cell culture-based vaccine [22]. The cell culture-based vaccine showed a significantly higher relative VE of 36.2% (95% CI : 26.1, 44.9), consistent with the primary analysis. It is essential to stress that the definition of ILI is decisive in these studies, as it may influence the results. Although the study found a concordance between ILI cases included in the study and influenza confirmed by the Centers for Disease Control and Prevention (CDC) laboratory, direct extrapolation is complicated because multiple circulating influenza-like illnesses follow the same seasonality as influenza, although influenza vaccines are only effective against influenza. It should also be noted that the allocation of vaccines should be random for both vaccines, so the penalization for not including PCR confirmation of influenza should be the

Table 1 **Characteristics of the studies comparing egg-based influenza vaccines and cell culture-based vaccines**

Type of study	First author, year of publication [reference]	Number of patients	Primary variable	Age group	Patients included	Effectiveness (percent) (95% CI)
Clinical trial	Frey S, 2010 [16]	11,404	ILI and PCR influenza confirmation	Patients 18-49	United States, Finland, and Poland	rVE versus placebo Cell culture-based trivalent: - 83.8 (61.0, 97.5) against vaccine strains - 69.5 (55.0, 97.5) against circulating strains Egg-based trivalent: - 78.4 (52.1, 97.5) against vaccine strains - 63 (46.7, 97.5) against circulating strains
Retrospective observational study	Klein NP, 2020 [21]	3,053,248	Influenza confirmed by PCR	Patients aged 4 to 64 years	Kaiser Permanente Members (Northern California, USA)	rVE against A virus (cell culture vs egg) 4-64 years: 8.0 (-10, 23) 4-17 years: 17.8 (-6.2, 36.4) 18-64 years: -5.8 (-36.1, -17.7) rVE against B virus (cell culture vs egg) 4-64 years: 39.6 (27.9, 49.3)[a] 4-17 years: 42.3 (28.4, 53.5)[a] 18-64 years: 21.4 (-36.1, 17.7)
	Boikos C, 2020 [22]	1,353,862	Visit to primary care for ILI	Patients older than 4 years	US Primary Care electronic medical records (EMR)	rVE (cell culture vs egg) 18- 64 years: 26.8 (14.1, 37.6) ≥ 65 years: -7.3 (-51.6, 24.0) 4-17 years: 18.8 (-53.9, 57.2)
	DeMarcus L, 2019 [18]	4,037	Influenza confirmed by PCR and/or culture	≥ 6 months	Outpatient records Department of Defense	rVE versus non-vaccinated All ages: - Non-significant differences for H3N2 - H1N1 86 (68, 91) for egg-based 61 (38, 76) for cell culture-based Children (6 months-17 years) - Non-significant differences for H3N2 - H1N1 88 (80, 93) for egg-based 56 (15- 77) for cell culture-based
	Bruxvoort KJ, 2019 [20]	8,132	Hospitalization for influenza confirmed by PCR	Patients > 4 years to < 65 Patients > 65 years	Kaiser Permanente Members (California, US)	rVE (cell culture vs egg) < 65 years: 43 (-45, 77) > 65 years: 61 (-63, 91)
	Izurieta HS, 2019 [23]	> 16,000,000	Primary: Hospital records (admissions + emergency)	Patients ≥ 65 years	Medicare members (US)	rVE (cell culture vs egg) vs hospitalizations All ages: - 11 (7.9, 14.0)
	Izurieta HS, 2020 [24]	12,777,214	Primary: Hospital records (admissions + emergency)	Patients ≥ 65 years	Medicare members (US)	rVE (cell culture vs egg) vs hospitalizations All ages vs hospital events: - 0.8 (-4.6, 5.9.) All ages vs hospitalizations - 3.4 (-3.6, 9.8)
	Izurieta HS, 2020, [25]	12,700,000	Primary: Hospital records (admissions + emergency)	Patients ≥ 65 years	Medicare members (US)	rVE (cell culture vs egg) vs hospitalizations All ages vs hospital events: - 2.8 (-2.8, 8.2)

[a] $p < 0.0001$; rVE: relative vaccine effectiveness; CI: confidence interval

same. Furthermore, the statistical analysis has some shortcomings. The distribution of patients receiving each vaccine is uneven, with older white people more frequently receiving the cell culture-based vaccine. Models for selecting variables based on statistical ratios were used when it would have been more appropriate to use a multivariate analysis. In conclusion, many factors of this study were not properly controlled.

Another comparative study of the 2 vaccines during the 2017-2018 season in the United States was performed by a US team led by Héctor Izurieta [23]. This was a retrospective study that included Medicare beneficiaries ≥ 65 years of age who had received an influenza vaccine (egg-based/cell culture-based quadrivalent, egg-based high-dose, adjuvanted, or standard-dose trivalent). The investigators analyzed the relative VEs of each type of vaccine in the prevention of influenza-related hospital events (without diagnostic conformation) including hospitalizations and emergency visits. The results showed that the quadrivalent cell culture-based vaccine was more effective than the standard-dose quadrivalent egg vaccine (relative VE 10%, 95% CI: 7, 13). However, there were no statistically significant differences with the high-dose trivalent vaccine, also produced in eggs (relative VE 11 % vs. 9%, respectively) [23]. It is important to note that influenza cases were not confirmed by PCR in this study. Moreover, the predominant A strain in the 2017-2018 season was H3N2, and there was no concordance with the B strain included in the trivalent vaccine. Another factor to consider with regard to the study methodology is the population included. Elderly people (≥ 65 years) are candidates for the high-dose vaccine as a first option. However, since the study took place in the US, clinicians selected the vaccine and the antigenic load to administer to patients. Although the analysis is correct from a statistical point of view, with an adjustment for previously measured confounders, the design does not allow adjustment for unmeasured confounders.

During the following season, 2018-2019, the team led by Izurieta *et al.* also conducted comparative research among vaccines following the same methodology as in their previous study [24]. The study, that included nearly 13 million Medicare beneficiaries over the age of 65, showed that egg-produced quadrivalent vaccines were more effective than cell culture-generated vaccines (relative VE 2.5 %; 95 % CI: -2.4, 7.3) although the results were not statistically significant [24].

The team led by Héctor Izurieta has recently published a new study comparing the effectiveness of influenza vaccines in the 2019-2020 season [25]. This study followed the same design as described in previous seasons [23,24], but added a quadrivalent recombinant HA-based influenza virus vaccine, delivering a high HA dose (45 μg per strain) [26]. In that season, the quadrivalent vaccine produced in mammalian cells was not significantly more effective than the egg-based vaccine (2.8 %, 95 % CI: -2.8, 8.2).

DISCUSSION

In the last two decades, the use of cell culture platforms for the production of influenza vaccine has been explored as an alternative to the generation of vaccines using fertilized chicken eggs. However, until now, the clinical effectiveness of these two vaccines has not been directly compared in clinical trials. In this critical review of the literature, we have analyzed studies published over the past 10 years comparing the effectiveness of the two vaccines, including 7 observational studies and a clinical trial in which both vaccines were compared with placebo. We conclude that there is insufficient evidence to show that the vaccine produced in cell culture is superior to that generated in egg.

The clinical trial developed by Frey et al. [16] was designed to demonstrate the efficacy of cell culture-based and egg-based vaccines separately against placebo. This approach was adopted to decrease the sample size required for the placebo group. However, this clinical trial was not designed to allow a direct comparison between the 2 types of vaccines. The European Medicines Agency (EMA) also recommends in its guidelines for the development of influenza vaccines that trials should be double-blind and include other secondary variables, such as hospitalization, all-cause mortality, all-cause pneumonia, and otitis (in children), factors not addressed in this study [27]. In brief, the results of this trial do not allow conclusions to be drawn on the efficacy of cell culture-based influenza vaccine compared with egg-based vaccine.

The results of the 7 observational studies included in our review, mostly conducted in the 2017-2018 influenza season and all in the U.S., are inconsistent. The relative VEs varied widely among these studies despite the fact that some were performed in the same population, and in some cases, using the same data records (Table 1). While some studies favored the cell culture-based vaccine, in others, the relative VE was better for egg-based vaccine. It should also be noted that many of the studies do not use ILI together with laboratory confirmation of influenza by PCR as their primary variable, as recommended by EMA for the development of influenza vaccines [27].

When the 3 studies carried out by the team of Izurieta *et al.* during 3 consecutive seasons (2017-2020), in which the same population was included and the same methodology followed, are compared, the results again lack consistency [23-25]: the effectiveness data for the 2017-2018 season favored cell culture-based vaccine, while in the following two seasons, a better trend was observed for egg-generated vaccines. In most cases the differences were not statistically significant, so no clinical conclusions can be drawn.

Differences on effectiveness of influenza egg-based vaccines versus cell culture-produced vaccines have been analyzed on previous reviews [28]. This study evaluated recent data regarding the effectiveness of both vaccines and concluded that the global results seem to support greater effectiveness, backed by greater antigenic stability of cell culture-derived vaccines over egg-based vaccines [28]. However, it is important to point out that the analysis that led to these conclusions were not mainly based on studies directly comparing both kinds of vaccines, but on individual effectiveness studies

that are difficult to compare due to the differences in their methodological designs.

Recently, 3 recognized scientific bodies have conducted a comprehensive review of the available evidence for new influenza vaccines. The European Center for Disease Prevention and Control (ECDC) has conducted a comprehensive evaluation of the quality of evidence using GRADE (Grading of Recommendations Assessment, Development, and Evaluation) methodology [29]. The following vaccines were analyzed in a systematic literature review: MF59®-adjuvanted vaccine, cell culture-based vaccine, high-dose vaccine, and recombinant vaccine. The ECDC concluded that cell culture-based vaccines are effective compared to non-vaccination. However, no superiority conclusions were established regarding the effectiveness of these vaccines compared to egg-based vaccines, as the available data are limited. The German Standing Committee on Immunization (STIKO) has also analyzed the current literature on influenza vaccines using GRADE methodology. Their paper concludes that the cell culture-based vaccine is likely to be effective when compared with placebo and that data comparing its effectiveness with that of egg-based vaccines are limited. The National Immunization Advisory Committee of Canada (NACI) has also issued a supplementary statement to the Canadian immunization guideline providing guidance on the use of standard-dose quadrivalent vaccine produced in cell cultures [30,31]. In this case, recommendations were based on a systematic review conducted according to the PRISMA criteria, following standard NACI methodology for grading evidence [32]. It concluded that there were no consistent or statistically significant results to confirm that the cell culture-propagated vaccine is more effective than egg-produced vaccine.

Based on all studies evaluated, it is impossible to establish differences in vaccine effectiveness between egg-based and cell culture-based vaccines, given the lack of significant differences. Furthermore, the results of multiple studies diverge. While some show the superiority of cell culture-propagated vaccines, others have found that egg-based vaccine is more effective. Methodological differences are among the factors that account for the inconsistent results. It seems clear that coherent study designs are desirable. Sample size should be larger to help achieve statistical significance between the different groups. Another variable to consider is the correct definition of ILI and the confirmation of influenza by PCR. Since the diagnosis of ILI is very heterogeneous and varies from specialist to specialist, failure to obtain PCR confirmation can generate significant biases. It should also be remembered that influenza vaccines are only effective against influenza disease.

Finally, another obvious factor is that the influenza virus is very complex and highly susceptible to variations. All of the studies evaluated a single season; however, the characteristics of the influenza virus and its infectious behavior must be followed up over multiple seasons in order to determine the real effectiveness of influenza vaccines.

Even though every effort was made to deliver a comprehensive review of the available literature using an exhaustive and significative literature search strategy, this review has limitations. We only report results from articles published in English or Spanish from the past 10 years. In addition, our results are limited by the quality of the identified studies, but it should be remembered that the objective of this review was in fact to analyze the published evidence. On the other hand, as noted previously, the results are bound by the inherent limitations of the studies included in the literature review such as outcomes, case definitions, mode of data collection and detection methods for laboratory-confirmed influenza. A timely meta-analysis/systematic review of this topic would provide stronger evidence and would help resolve possible divergences in the literature.

CONCLUSION

Based on the studies evaluated, no differences can be established in the effectiveness of the cell culture-based and the egg-based influenza vaccines. Independent analyses conducted by NACI, ECDC and STIKO also reached the same conclusion. Future studies should take into account the high complexity of the influenza virus and be designed by consensus, with a larger sample size, precise definition of ILI, including PCR confirmation of influenza, and, above all, follow-up over multiple seasons to determine superiority in vaccine effectiveness.

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CONFLICT OF INTEREST

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