Influenza vaccine outcomes: a meta-analysis revealing morbidity benefits amid low infection prevention

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We need to shift the vaccination message to emphasise infection prevention and protection against severe outcomes. The emphasis on flu vaccination should be on preventing severe illness *versus* infection. https://bit.ly/3ZR6Nl7

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Abstract

Background The morbidity and mortality associated with influenza viruses are a significant public health challenge. Annual vaccination against circulating influenza strains reduces hospitalisations and increases survival rates but requires a yearly redesign of vaccines against prevalent subtypes. The complex genetics of influenza viruses with high antigenic drift create an ongoing challenge in vaccine development to address dynamic influenza epidemiology. Understanding the evolution of influenza viruses and the vaccine's effectiveness against different types and subtypes is pivotal to designing public health measures against influenza.

Methods We conducted a systematic review and meta-analysis of 192 705 patients, collecting information on the incidence and severity of the disease. The results of this meta-analysis were further validated using data from 6 594 765 patients from TriNetX. We analysed the prevalence of the most common influenza A virus (IAV) subtypes (H1N1 and H3N2) and influenza B virus (IBV), as well as vaccination effectiveness against them in three age groups, given that age is associated with influenza disease severity.

Results Our analysis reflects that overall vaccination against H1N1 IAV and IBV is effective in reducing infection and influenza-related complications in children aged <5 years old, individuals between 5 and 65 years old and older adults aged >65 years old. By contrast, while vaccination against H3N2 IAV is effective in protecting against infection in infants <5 years old, it provides reduced protection against infection in older individuals.

Conclusions Despite higher infection rates, vaccination against H3N2 remains as highly effective as vaccination against H1N1 and IBV in reducing influenza-related morbidity and mortality in all age groups. Detailing vaccine effectiveness in terms of infection protection and disease burden across different age groups is necessary for understanding vaccine impacts in terms of other outcomes, *e.g.* hospitalisations, mortality and disease severity; for improving vaccine formulations and public awareness; and for enhancing vaccination campaigns to improve coverage and public acceptance.

Introduction

Human influenza viruses are classified into three main types: influenza A (IAV), influenza B (IBV) and influenza C (ICV). Each group is further categorised into subtypes or lineages based on the surface

proteins haemagglutinin and neuraminidase. These surface proteins are crucial for the virus's ability to infect and replicate within cells, and they serve as the primary targets in vaccine design.

The predominant seasonal subtypes of IAV in humans are H1N1 and H3N2. Additionally, IBV is a common pathogen that infects humans. IBV has been categorised into two main lineages: Victoria and Yamagata [1]. These viruses are included in the influenza vaccine formulation based on their clinical significance.

Vaccination against different influenza viruses reduces the possibility of infection and pathogenesis and the risk of influenza-related complications, including hospitalisation and death, predominantly among high-risk groups such as older adults, young children, pregnant women and immunocompromised individuals [2].

Influenza viruses are antigenically highly variable, which demands annual vaccine updates to maintain their effectiveness [3]. The incidence of different influenza viruses also varies globally from year to year, presenting an uncertainty factor in terms of vaccine production and logistics. Annual influenza vaccination is recommended in groups with a risk of complication and a high exposure potential, such as children from 6 months to 5 years old, adults aged >65 years old, pregnant women, patients with chronic diseases and healthcare workers [4]. The seasonality of circulating influenza viruses results in a concentration of cases in winter, stressing healthcare systems [5]. Preventive vaccination alleviates overcrowding in healthcare centres and reduces hospitalisation costs [6]. Despite the high vaccination rate against influenza in general, population herd immunity is not achieved, although some studies have described it is possible to reach this state in specific centres for risk groups at nursing homes [7, 8].

Analysis of influenza vaccine effectiveness against different influenza viruses is crucial for better surveillance, vaccine development and public health preparedness. Because age is a primary factor associated with influenza disease severity, especially in newborns and older adults, analysing vaccine effectiveness across different age groups is important for influenza vaccination improvements to reduce disease burden.

Here, we performed a systematic review and meta-analysis, collecting information on the incidence and severity of the disease from articles covering patients' data between 2003 and 2023. This information was used to prepare a dataset based on published literature. Using TriNetX (https://trinetx.com), we selected a second dataset to compare and validate the results from the published literature dataset. Both the literature dataset and the TriNetX dataset were used to analyse the prevalence of the most common IAV subtypes (H1N1 and H3N2) and IBV, as well as the influence of vaccination in different age groups. The analysis emphasises the importance of vaccine protection against influenza complications beyond their role in protection from infection. Public awareness on this matter may improve influenza vaccination acceptance.

Materials and methods

This study followed the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) statement [9]. The research protocol was registered at the International Prospective Register of Systematic Reviews (PROSPERO) (#CRD 42024532743).

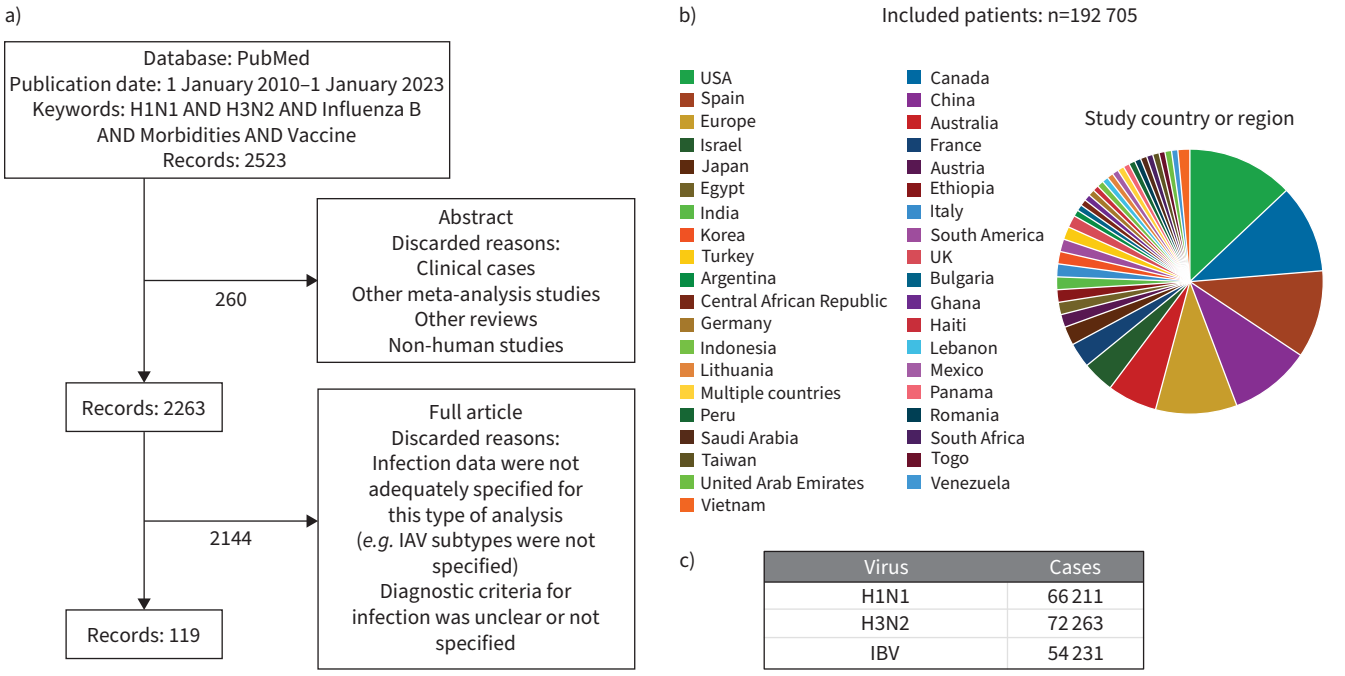
A systematic review and meta-analysis were conducted in accordance with the PRISMA guidelines [9] to characterise the current burden of infections and outcomes in patients with a laboratory-confirmed diagnosis of IAV and IBV over the last two decades (2003–2023). The inclusion of articles was limited to studies that had a reverse transcription (RT)-PCR laboratory confirmed diagnosis for the different influenza viruses. Search criteria included patients of all ages, regardless of comorbidities, treated in all types of healthcare centres, of all genders and with or without risk factors. Reasons for exclusion included animal studies; viral, fungi or parasitic co-infections; duplicate studies; single-patient studies; and studies with partial or insufficient information.

Systematic scientific literature research

The literature used in the meta-analysis was obtained through a systematic search of international database PubMed, filtered by articles containing observational studies with RT-PCR-confirmed influenza cases between 2003 and 2023. Keywords used to narrow the search range included "H1N1 AND H3N2 AND Influenza B AND Morbidities AND Vaccine". Only articles written in English were included.

Selection criteria

Articles obtained in the previous step were selected after reading the title and the abstract, evaluating them individually and discarding duplicate articles (two independent readers). The full text of the selected articles was read and the most suitable publications were included, as detailed in figure 1a. Selected articles included



Virus	Cases
H1N1	66 211
H3N2	72 263
IBV	54 231

FIGURE 1 The selection process of the studies included in the meta-analysis and primary data analysis. a) Schematic representation of the selection process, with 119 studies included in the meta-analysis. b) The total number of patients included in this study (n=192 705) and a pie chart of the percentage of studies from each country. c) Number of cases for each virus. These data were used to build the literature dataset. IAV: influenza A virus; IBV: influenza B virus.

patients tested for influenza infection by RT-PCR diagnosis in a laboratory. Because articles were authored by hospital-affiliated groups, we assumed that data from all patients were collected in a hospital, but articles did not mention if the patients were hospitalised or discharged with symptomatic treatment.

Extraction of the information

The relevant information of each publication was collected and added to a Microsoft Excel document (supplementary table S1), including the author, the country where the study was performed, the year of the study, the total number of patients studied, the age of the patients, the number of patients with confirmed influenza and the number of uninfected patients (negative result in RT-PCR) used as control. This literature dataset also contained the number of influenza-vaccinated patients who became infected by any influenza virus during the first year after vaccination, as well as the corresponding controls of unvaccinated patients enrolled that year. The influenza virus subtypes H1N1, H3N2 and IBV were also considered in the analysis.

R script/data treatment

R v3.6.0 was used to process the literature dataset data, using meta4.11-0, metafor2.1-0 and dmeter0.0.9000 library packages to assist in the meta-analysis. The variation between the studies and the population was studied and corrected using a random effects approach [10].

The analysis included the variables age, vaccination status, infected or not infected, and influenza virus type or subtype.

Model validation using the TriNetX online tool

The meta-analysis based of the literature dataset was validated using the TriNetX database (TriNetX's Research Network, https://trinetx.com) that gathers information on patients hospitalised with influenza. This tool allows the screening of patients by real-time querying. It also allows the study of different cohort outcomes within an established period of time. The data obtained from TriNetX have been named the TriNetX dataset for the purposes of this study.

The TriNetX dataset used in this study was collected in March 2024 from the TriNetX Global Collaborative Network, which provided access to electronic medical records (diagnoses, procedures,

medications, laboratory values, genomic information) from approximately 90 million patients from 73 healthcare organisations. TriNetX, LLC, is compliant with the Health Insurance Portability and Accountability Act (HIPAA), the US federal law that protects the privacy and security of healthcare data, and any additional data privacy regulations applicable to the contributing healthcare organisation. TriNetX is certified to the ISO 27001:2013 standard and maintains an Information Security Management System to ensure the protection of the healthcare data to which it has access to and to meet the requirements of the HIPAA Security Rule. Any data displayed on the TriNetX Platform in aggregate form, or any patient level data provided in a dataset generated by the TriNetX Platform, only contain de-identified data as per the de-identification standard defined in Section 164.514a of the HIPAA Privacy Rule. The process by which the data are de-identified is certified through a formal determination by a qualified expert as defined in Section 164.514b1 of the HIPAA Privacy Rule. Because only de-identified patient records were used in this study, and the study did not involve the collection, use or transmittal of individually identifiable data, this study did not require institutional review board approval.

The overall cohorts included all patients who were diagnosed with influenza viruses and certified by laboratory tests (using Logical Observation Identifiers Names and Codes (LOINC) identifiers). In particular, tests included RNA presence detection by nucleic acid amplification with probe or non-probe in nasopharynx and lower respiratory specimens or in isolates. In this TriNetX dataset, cohorts were built according to IAV subtypes H1N1 and H3N2, as well as IBV. The codes selected for each cohort are summarised in the TrinetX cohort codes in the supplementary material. Each cohort was then subdivided into vaccinated and unvaccinated groups, by adding to the query the codes for the different influenza virus vaccines, including intranasal, intramuscular or intradermal trivalent (laiv3 or iiv3, respectively) vaccine (derived or not from cells), trivalent vaccine derived from recombinant DNA (riv3), intranasal or intramuscular quadrivalent (laiv4 or iiv4, respectively) vaccine (derived from tissue culture or chicken embryonated eggs), intramuscular inactivated (iiv) vaccine, and all variants including adjuvants or not. The codes used for the acquisition of vaccinated subgroups were the same in the three cohorts (H1N1, H3N2 and IBV). The same codes were used for the vaccinated group, for which patients must have been vaccinated with any of those vaccines, and the unvaccinated group, for which patients were not vaccinated with any vaccine. The TriNetX dataset was further used to analyse age and comorbidity as factors influencing vaccine protection.

Statistical analysis

For statistical analysis, IBM SPSS statistics v22 software was applied. The measurement data were expressed as mean±SD and compared using an independent t-test, while the categorical data were expressed as n (%) and compared by χ^2 or Fisher exact test with continuity correction. Only p-values <0.05 were considered statistically significant. RevMan software v5.4.1 was used to assess the heterogeneity of the literature to draw forest plots.

Results

A total of 119 articles from 39 countries were chosen after an initial examination of 2523 studies that reported the outcomes of hospitalised patients and from primary healthcare centres with laboratory-confirmed IAV or IBV infection. The selection criteria discarded redundant studies, clinical cases, other meta-analyses or reviews and animal studies (figure 1a). The articles chosen for further analysis included 192 705 patients of any age. Figure 1b and supplementary table S1 show the geographic origin of these studies. Raw data included into this literature dataset were initially divided into different groups according to the influenza virus types or subtypes H1N1, H3N2 and IBV (figure 1c).

Prevalence ratio of IAV and IBV across age groups

Children <5 years old and older adults >65 years old represent the primary age demographics diagnosed with influenza. To elucidate the impact of H1N1 and H3N2 IAV subtypes and IBV within the population, the prevalence in age groups <5 years old and >65 years old was calculated and compared to the rest of the population. No significant differences in the prevalence of any of the viruses was observed in the age group <5 years old compared to patients >5 years old (figure 2a, top panel). A meta-analysis using the literature dataset was also conducted to assess the odds of infection of each virus among children <5 years old and adults >65 years old compared with the rest of the population in each case. Complete information about the random forests and quality controls of funnel plots are summarised in supplementary figure S1.

The odds of IAV infection among patients <5 years old (figure 2b, top panel) did not exhibit significant differences compared to those >5 years old for H1N1-infected patients (odds ratio (OR) −0.24, 95% CI

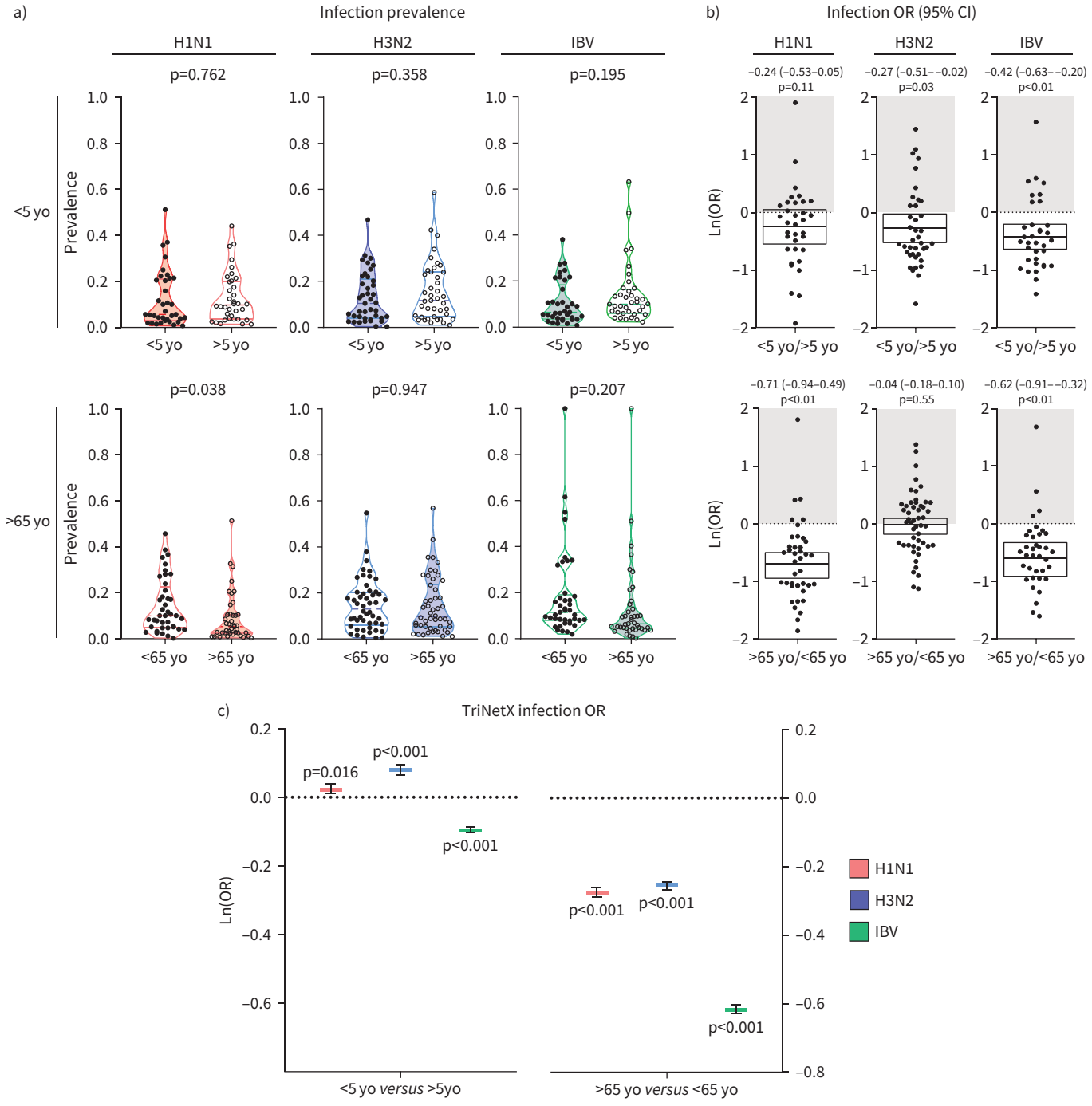


FIGURE 2 Prevalence of influenza subtypes and odds ratio (OR) of infection in three age groups. a) Prevalence of influenza A virus subtypes H1N1 and H3N2 and influenza B virus (IBV) in patients <5 years old *versus* >5 years old, and >65 years old *versus* <65 years old. Results were obtained by random forest meta-analysis, and each point represents a single study. This analysis was performed using the literature dataset. b) Ln(OR) of infection was calculated for each influenza virus type or subtype. In the top panels, patients >5 years old were used as controls, whereas in the bottom panels, patients <65 years old were used as controls (shaded in panel a). This analysis was performed using the literature dataset. c) Infection Ln(OR) of patients <5 years old *versus* >5 years old, and patients >65 years old *versus* <65 years old, both obtained using the TriNetX online tool. The dotted line represents the reference Ln(OR) corresponding to the patients >5 years old (left panel) and <65 years old (right panel). This analysis was performed using the TriNetX dataset. yo: years old.

−0.53 to 0.05, p=0.11) and H3N2 patients (OR −0.27, 95% CI −0.51 to −0.02, p=0.03). However, in the case of IBV infection, ORs were significantly lower in children <5 years old (OR −0.42, 95% CI −0.63 to −0.20, p<0.01).

A higher prevalence for H1N1 was observed among patients <65 years old compared to >65 years old (0.1433 *versus* 0.0905, p=0.0384), but the difference was nonsignificant for H3N2 (0.1469 *versus* 0.1454, p=0.947) or IBV (0.1852 *versus* 0.1326, p=0.207) (figure 2a, bottom panel). The meta-analysis ORs using the literature dataset indicated a higher incidence of infections among patients <65 years old compared to those >65 years old for IAV subtype H1N1 (OR 0.71, 95% CI −0.94 to 0.49, p<0.01) and IBV (OR −0.62, 95% CI −0.91 to −0.32, p<0.01) (figure 2b, bottom panel). Conversely, IAV subtype H3N2 (OR −0.04, 95% CI −0.18 to 0.10, p=0.55) exhibited no statistically significant OR differences between the two age groups.

To corroborate these findings, the TriNetX online tool was used for an independent assessment comparing the high-risk groups (<5 and >65 years old) (figure 2c). The gathered information was used to generate the TriNetX dataset and initially used to validate the results from the analysis of the literature dataset. A Ln(OR) x=0, indicated by the dotted line, represents no significant differences between groups. Children <5 years old exhibited a slightly higher odds for H1N1 (OR 0.021, 95% CI 0.004 to 0.041, p=0.016) and H3N2 (OR 0.079, 95% CI 0.064 to 0.097, p<0.001) infections, as well as slightly lower odds for IBV (OR −0.097, 95% CI −0.102 to −0.087, p<0.001), consistent with the trends observed in the meta-analysis. Conversely, the population >65 years old exhibited significant lower odds for all three influenza viruses: H1N1 IAV OR −0.276, 95% CI −0.292 to −0.259, p<0.001; H3N2 IAV OR −0.251, 95% CI −0.267 to −0.244, p<0.001; IBV OR −0.620, 95% CI −0.620 to −0.602, p<0.001.

Effectiveness of influenza vaccination against infection caused by influenza viruses

One possible explanation for the lower odds of infection in individuals >65 years old could be the higher vaccination rates in this age group. Therefore, we analysed the impact of influenza vaccination on the prevalence of being influenza-positive based on RT-PCR diagnosis and odds (influenza virus RT-PCR positive *versus* the total number of individuals included in the studies used for this literature database) across all ages. For this, influenza-infected patients were divided by the kind of influenza virus (H1N1, H3N2 or IBV), and each subtype subdivided into vaccinated or unvaccinated. The prevalence was then recalculated in the overall population based on vaccination status.

In the meta-analysis performed with the literature dataset (figure 3a), IAV H1N1 infections had a significantly lower prevalence in the vaccinated group (0.1242) compared to the unvaccinated (0.2203, p<0.01). Similarly, IBV prevalence decreased from 0.2322 in the unvaccinated group to 0.1445 in the vaccinated group (p=0.004). However, there was no significant reduction in the prevalence of H3N2, with prevalence rates of 0.2414 in the unvaccinated group and 0.2081 in the vaccinated group (p=0.534). Complete information about the random forests and quality controls of funnel plots for vaccination are summarised in supplementary figure S2.

The infection OR analysis for each subtype of influenza virus, as depicted in the meta-analysis (figure 3b), illustrates the effectiveness of protection from infection in the context of vaccination. Notably, vaccination demonstrates superior protection against IAV H1N1 infection (Ln(OR) −0.77, 95% CI −0.88 to −0.65, p<0.01). Similarly, vaccination exhibits effectiveness against IBV infection (Ln(OR) −0.73, 95% CI −0.90 to −0.55, p<0.01). Vaccination also presents efficacy against H3N2 with a significant reduction in diagnosed infections (Ln(OR) −0.27, 95% CI −0.37 to −0.16, p<0.01), although this reduction was lower compared to the protection observed against H1N1 IAV and IBV.

The TriNetX patient dataset was again used to validate the meta-analysis performed with the literature dataset. In addition, influenza vaccination effectiveness was considered in <5, >5–<65 and >65 years old subgroups (figure 3c), compared to the total population. When all ages were included, vaccination was effective against all virus tested (figure 3b). The analysis using TriNetx dataset showed a similar profile for the three influenza viruses as observed in the metanalysis using the literature dataset. However, when an aged-based analysis was performed, influenza vaccination was highly effective against infection by the three influenza viruses in children <5 years old (Ln(OR) −0.432±0.11, p<0.001 for H1N1; −0.362±0.08, p<0.001 for H3N2; and −0.467±0.06, p<0.001 for IBV). For patients >5 years old, and in particular in older adults (>65 years old), vaccine protection was effective against H1N1 and IBV (Ln(OR) −0.197±0.10, p<0.001 for H1N1; and −0.095±0.05, p<0.001 for IBV). However, vaccination protection from infection was less effective against H3N2 (blue bars), where vaccination showed no significant differences for the 5–65 years old group (Ln(OR) 0.013±0.04, p=0.49), and vaccinated patients >65 years old had even more infections than non-vaccinated patients in the same age group (Ln(OR) 0.144±0.08, p<0.001). In general, vaccinated children were more protected against any influenza virus infection than the other age groups. In vaccinated children <5 years old, infection with IBV was less probable than infection with IAV subtype H1N1 (Ln(OR) −0.432±0.11, p<0.001 *versus* −0.467±0.06, p<0.001).

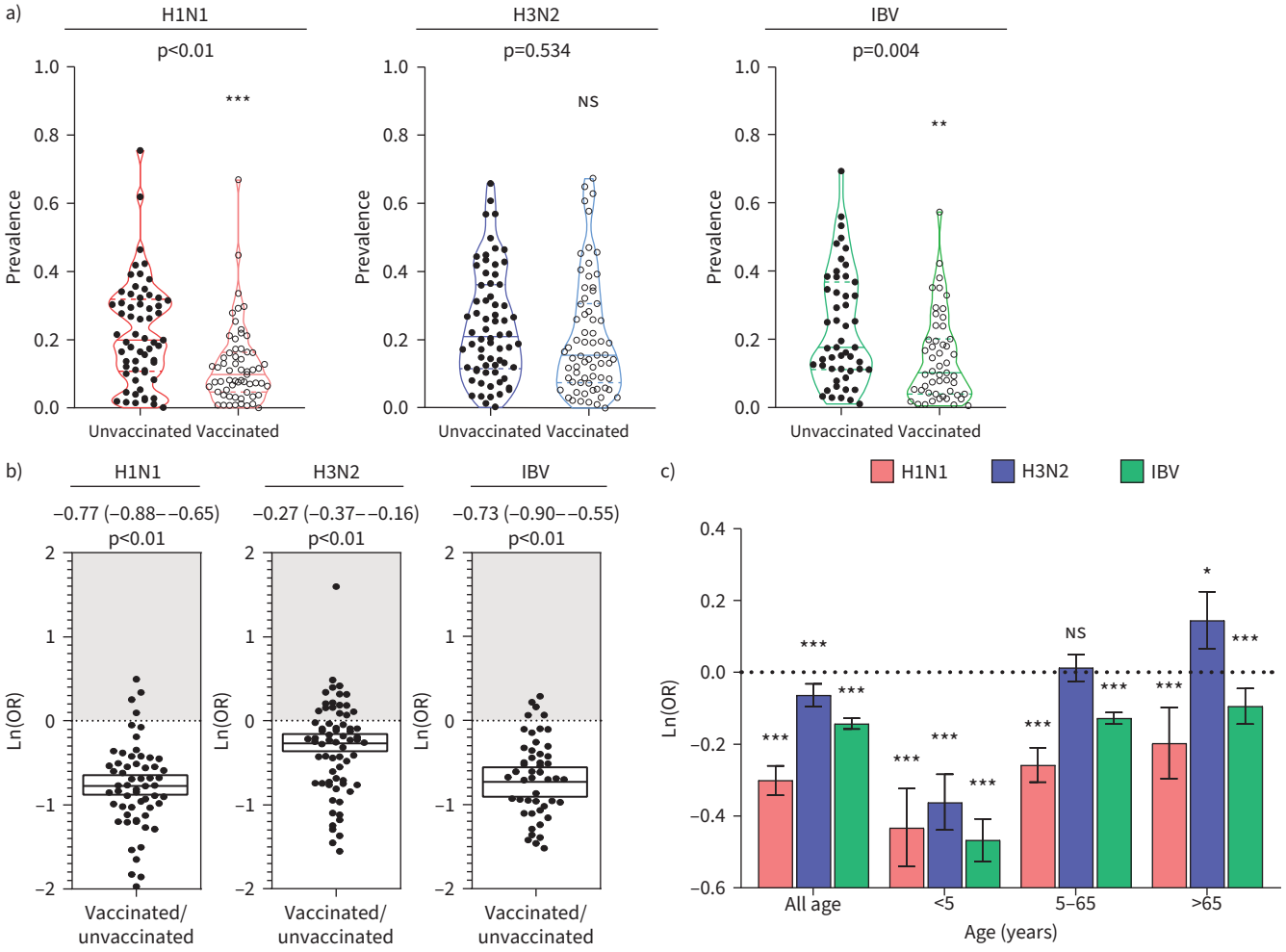


FIGURE 3 Vaccine effectiveness in preventing infections diagnosed by reverse transcription-PCR. a) Violin plots showing the prevalence (ratio) of influenza A virus (IAV) subtypes H1N1 and H3N2 and influenza B virus (IBV) in vaccinated *versus* unvaccinated cohorts from the total number of individuals included in the studies used in the meta-analysis cohort across all ages. Each point represents a study. The median (solid lines) and the quartiles (dotted lines) are shown. This analysis was performed using the literature dataset. b) Ln odds ratio (OR) of vaccinated *versus* unvaccinated cohorts, comparing vaccine effectiveness depending on the three influenza viruses under analysis. This analysis was performed using the literature dataset. c) Ln(OR) of IAV subtypes H1N1 and H3N2 and IBV in vaccinated *versus* unvaccinated patients in hospitalised cohorts obtained from the TriNetX online tool and the TriNetX dataset. Patients were subdivided into the three indicated ages groups. *: p<0.05; **: p<0.01; ***: p<0.001.

Influenza vaccination effectiveness in reducing mortality associated with IAV and IBV infections

Vaccination effectiveness is usually measured in terms of prevention from laboratory-confirmed infection, as just analysed. Assessing the effectiveness of vaccines in reducing the mortality of the disease associated with infection is challenging and can only be performed when the vaccination has been well implemented and a sufficient number of cases can be gathered over time. The impact of vaccines in preventing death associated with influenza virus-diagnosed infections was assessed using the TriNetX dataset due to the lack of sufficient data in the studies in the literature dataset used for meta-analysis. The effect of influenza vaccination in preventing mortality in influenza-infected patients was initially assessed without differentiating among subtypes (figure 4a), by obtaining the influenza OR ratio. Longitudinal analysis considering the time from the medical report date of infection was also considered. The major outcome of interest was the mortality of influenza, labelled as “deceased” in TriNetX. The outcome “co-infection” was also studied because the relevance of bacterial infections in influenza-associated mortality has previously been demonstrated [11]. Co-infection was assessed by exploring bacterial lung infection as the input of the outcome analysis. Vaccination demonstrated significant effectiveness in mortality reduction, which gradually reduced from the initial month up to almost 1 year after infection.

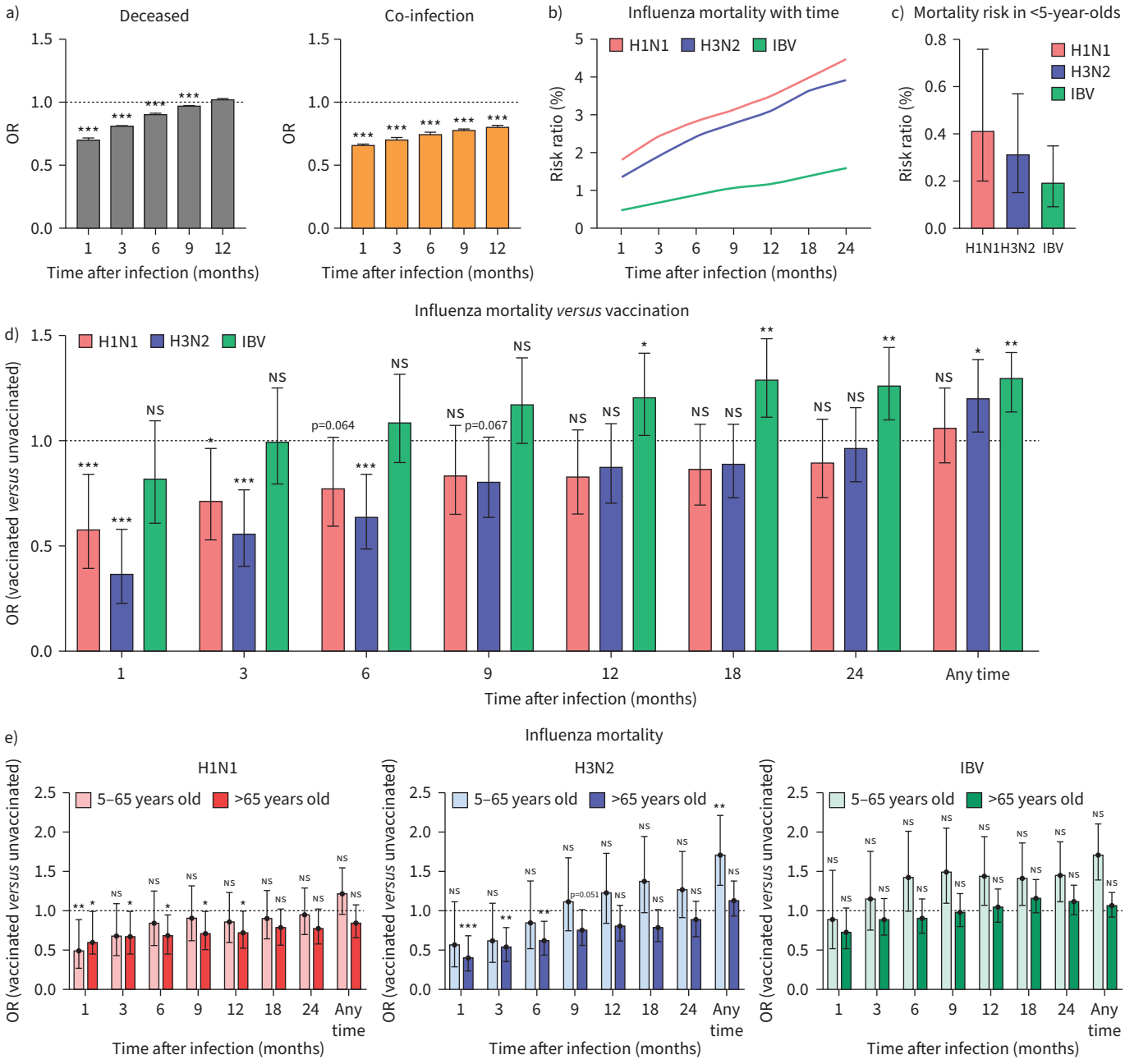


FIGURE 4 Vaccine effectiveness to prevent influenza virus infection-associated mortality obtained from the TriNetX dataset. a) Cumulative odds ratio (OR) of mortality after influenza virus infection (left panel) and cumulative OR of secondary bacterial infection as an outcome in infected patients after vaccination (right panel) *versus* time post infection. b) Mortality risk ratio (%) in the whole population (left panel) of the TriNetX dataset at different months after infection by different influenza viruses. c) Cumulative risk ratio (%) of mortality during the first year after influenza infection in patients <5 years old. d) Cumulative OR of mortality in influenza A virus (IAV) subtypes H1N1 and H3N2 and influenza B virus (IBV) infected patients after vaccination or no vaccination at different times post infection. e) Cumulative OR of mortality in patients infected with IAV subtypes H1N1 (red) and H3N2 (blue) and IBV (green), comparing subgroups of ages 5–65 years old and >65 years old, comparing mortality OR in vaccinated *versus* unvaccinated individuals at different times post infection. *: p<0.05; **: p<0.01; ***: p<0.001.

The impact of H1N1, H3N2 and IBV on influenza-related mortality was analysed by calculating risk of death associated with diagnosed cases of H1N1, H3N2 or IBV (figure 4b) using the TriNetX dataset. The analysis indicated that H1N1 poses the highest mortality risk ratio followed by H3N2 and IBV with the lowest risk. The mortality risk ratios were 1.8% for H1N1, 1.5% for H3N2 and 0.49% for IBV at 1-month post-infection, and 2.5% for H1N1, 1.9% for H3N2 and 0.7% for IBV at 3 months post-infection. Whilst

the overall slopes exhibited a parallel trend, during the initial 3 months, the slopes of the cumulative infection risks were slightly more pronounced for H1N1 and H3N2 compared to IBV. Children <5 years old (figure 4c) showed higher mortality when infected by H1N1 (risk ratio 0.41%, 95% CI 0.20% to 0.76%) than by H3N2 (risk ratio 0.31%, 95% CI 0.15% to 0.57%) and by IBV (risk ratio 0.19%, 95% CI 0.09% to 0.35%). However, the risk ratio of mortality in children during 1 year follow-up (figure 4c) after infection was on average 7-fold lower than that of the total population at the same time (figure 4b). This low risk, together with low number of cases in the TriNetX dataset, makes it difficult to further analyse this age group in the presented analysis.

As compared to the infection prevalence in influenza-vaccinated patients *versus* non-vaccinated patients (figure 3), the OR for death in vaccinated patients showed similar vaccination effectiveness again IAV. Influenza vaccination significantly decreased the mortality OR associated with H1N1 infection (figure 4d, red bars) even 3 months after the infection date (after 1 month: OR 0.57, 95% CI 0.39 to 0.84, p<0.001; after 3 months: OR 0.72, 95% CI 0.53 to 0.96, p=0.0271). No significant difference between vaccinated and non-vaccinated patients was detected after that, although there was a consistent trend in all data analysed showing OR mean values <1 even 24 months after infection. Influenza vaccination also significantly decreased mortality associated with infections by the H3N2 subtype even 9 months after infection (OR 0.64, 95% CI 0.49 to 0.84, p=0.067). Analysis of the TriNetX dataset indicated that influenza vaccination had a low impact on death associated with IBV infection (figure 4d, green bars) with OR 0.81 (95% CI 0.61 to 1.09, p>0.175) at 1 month post-infection. In this case, vaccination could be seen as less effective in preventing IBV mortality. However, IBV also presents a lower risk than IAV (figure 4b, c).

The effectiveness of influenza vaccination in preventing death in a patient who has been diagnosed with influenza was analysed in the intermediate age (5–65 years old) and older adult (>65 years old) groups (figure 4e), using the same TriNetX dataset. Influenza vaccination effectiveness in children <5 years old was not analysed because no deaths were recorded among vaccinated patients in this age group during the first year after the infection was diagnosed. The risk of death in unvaccinated children <5 years old is shown in figure 4c.

Interestingly, patients >65 years old presented a pattern of lower mortality OR values for H3N2 and IBV than patients between 5 and 65 years old. Furthermore, influenza vaccination presented a significant death OR reduction in patients >65 years old diagnosed with H1N1 or H3N2, even at 12 months post-vaccination for H1N1 (OR 0.723, 95% CI 0.523 to 0.998, p=0.0475) or 6 months post-vaccination for H3N2 (OR 0.616, 95% CI 0.439 to 0.863, p=0.0045). Analysis of the selected TriNetX dataset indicated a lower trend in patients >65 years old, but no significant effects of influenza vaccination in mortality OR value reduction in patients diagnosed with IBV.

Prevention of death-associated comorbidities through influenza vaccination

Certain coexisting conditions can affect influenza-related risks, including mortality risk. Influenza vaccinations has been described to reduce such potential risks [12, 13]. Seasonal influenza vaccine effectiveness surveillance [14] has been proposed as an epidemiological tool to better address influenza disease in terms of reducing morbidities and mortality and not just preventing infections.

The influence of pre-existing conditions on the mortality associated with H1N1, H3N2 and IBV infections in the context of influenza vaccination was analysed using our TriNetX dataset cohort, because the number of articles that included comorbidities and vaccination was limited in the literature dataset. The TriNetX dataset pre-existing condition details are available in supplementary table S2. The OR for mortality within the first 3 months after H1N1, H3N2 or IBV infection was analysed in the context of different pre-existing conditions (figure 5a).

Several comorbidities result in a significantly increased risk of mortality associated with H1N1 and H3N2 but this was less prominent for IBV infections. Potential cardiovascular conditions defined by TriNetX as musculoskeletal system or connective tissue diseases showed the highest risk after infection with IAV (H3N2 OR 6.46, 95% CI 5.53 to 7.56, p<0.001; H1N1 OR 5.15, 95% CI 4.43 to 5.99, p<0.001). Patients with neoplasms, mental disorders and diseases of the circulatory or genitourinary system were also more susceptible to the lethal effects of influenza (higher mortality ORs) than healthy individuals when unvaccinated. Vaccination significantly reduced the odds of mortality in patients with these comorbidities, although with some differences depending on the influenza virus infection. For instance, the odds of mortality with musculoskeletal system and connective tissue diseases decreased from 5.15 (95% CI 4.43 to 5.99) to 3.1 (95% CI 2.26 to 4.25) for H1N1, and from 6.46 (95% CI 5.53 to 7.56) to 1.9 (95% CI 1.37 to 2.6) for H3N2. Influenza vaccination was associated with a significant reduction in the OR for mortality in

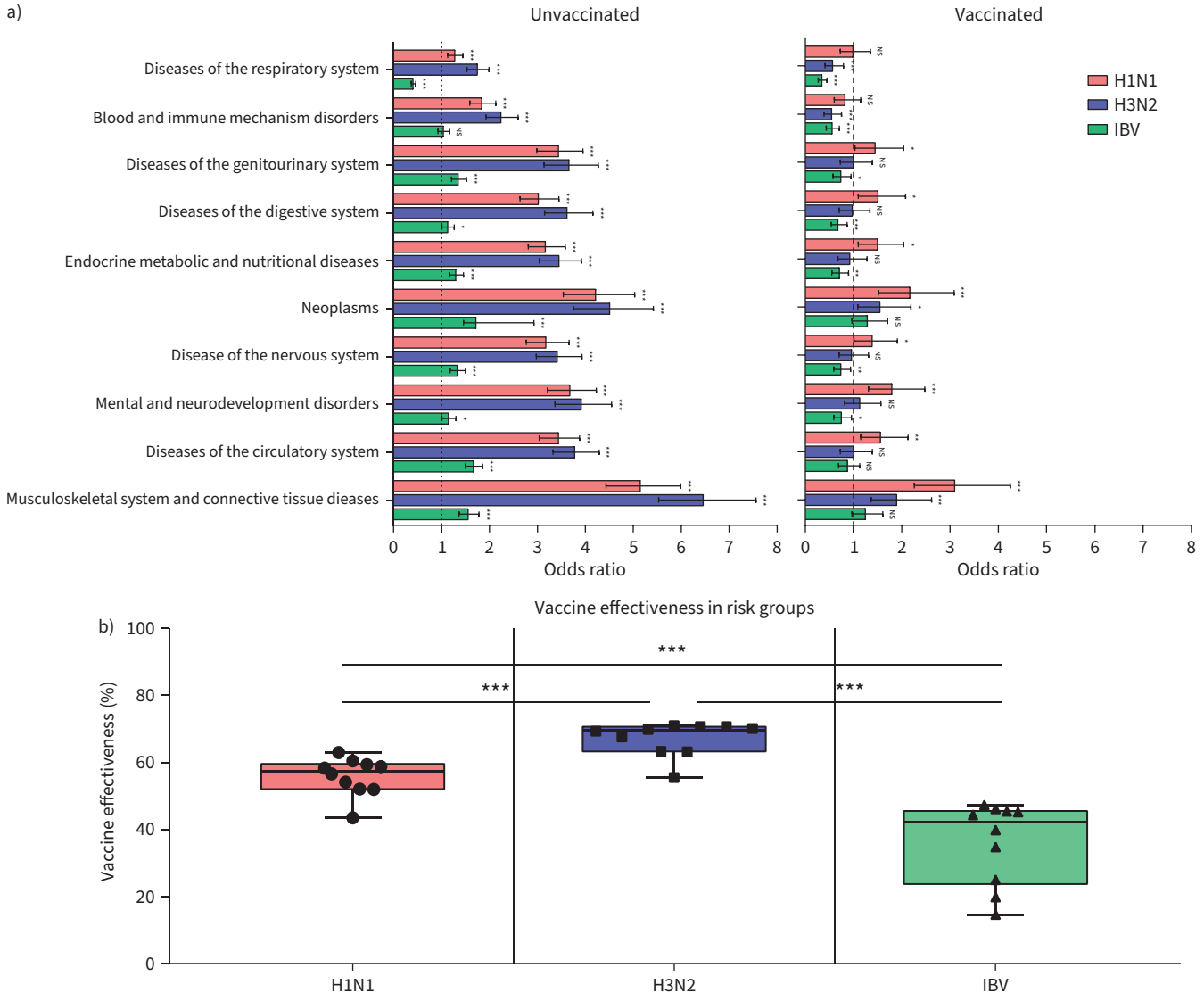


FIGURE 5 Odds ratio of mortality-associated risk factors of patients within the first 3 months after influenza diagnosis divided by influenza A virus (IAV) H1N1 and H3N2 and influenza B virus (IBV) and vaccine effectiveness (%). a) Odds ratio of mortality in unvaccinated and vaccinated patients with influenza who had previously had a certain condition within the indicated group of diseases. Analysis performed using the TriNetX dataset. b) Vaccine effectiveness per influenza type or subtype according to the capacity to prevent mortality in the risk groups mentioned in a. Each dot represents the vaccine effectiveness for each risk group mentioned in a. Error bars represent the 95% CI. *: p<0.05; **: p<0.01; ***: p<0.001.

patients with comorbidities such as mental disorders, diseases of the genitourinary system and diseases of the circulatory system. In most cases, influenza vaccination completely eliminated the increasing mortality risk of having certain comorbidities in patients diagnosed with H3N2 and in some cases H1N1 viruses.

The effectiveness of the influenza vaccine can be specifically analysed by calculating its impact on reducing mortality among patients diagnosed with influenza who belong to high-risk groups with pre-existing conditions. To estimate the influenza vaccination effectiveness in this scenario, we calculated the difference of normalised ORs for each risk group in vaccinated *versus* unvaccinated patients diagnosed with H1N1, H3N2 or IBV, and represented this in a single point as a percentage (figure 5b) using the following formula.

$$\text{Vaccine effectiveness } (\%) = \frac{\text{OR(unvaccinated)} - \text{OR(vaccinated)}}{\text{OR(unvaccinated)}} \times 100$$

The analysis of influenza vaccination in this context presents 67.08% effectiveness against the risk of death in the case of H3N2 infection. H1N1 showed 55.76% effectiveness, and IBV was the least effective with 36.25% effectiveness.

Discussion

The analysis presented here focuses on the effectiveness of influenza vaccination against H1N1 IAV, H3N2 IAV and IBV infections in three age groups (<5 years old, 5–65 years old and >65 years old) by analysing the effects of vaccination in preventing infections and death. This analysis was performed with an initial meta-analysis using a dataset from published literature. The results obtained from this meta-analysis were compared with a different dataset built from the TriNetX webserver to test initial conclusions. Despite some differences, both datasets provided comparable results upon analysis of the same queries.

The initial analysis of prevalence and infection ORs indicated a similar prevalence of IAV infections among children <5 years old compared to other age groups, while IBV was less common in this age group, possibly due to underdiagnosed milder infections. A similar trend was observed when analysing the TriNetX dataset. Individuals >65 years old exhibited significantly lower infection ORs for H1N1 and IBV in the literature meta-analysis as compared with H3N2 infections (figure 2b). By contrast, when using the TriNetX dataset, all influenza virus infections had lower ORs. Discrepancies between the meta-analysis and TriNetX data may be explained by the increased number of patients in TriNetX cohorts. Another possible difference between the two datasets is that we know the time span of the literature dataset but not that of the TriNetX. In this case, the data shared from the hospitals contributing to TriNetX do not include this detail.

The different impact of influenza infections in the <5 *versus* >65-year-old groups may appear counterintuitive, given the heightened propensity for hospitalisation and complications observed within older adults. Despite the expected higher vulnerability of the older adults, countries with influenza vaccination programmes show higher vaccination rates (65–80%) among adults >65 years old compared to other age groups (10–40%) [15, 16], potentially explaining the reduced infection prevalence in this demographic group.

Despite the effectiveness of influenza vaccination in protecting from infection, there is a potential source of bias in the subset of the infected population who may be asymptomatic or experience mild symptoms not requiring hospitalisation. Also, the number of false-negative tests for influenza is high, even in adults with community-acquired pneumonia requiring hospitalisation [17]. Considering the high annual vaccination rates, it is likely that the number of non-hospitalised vaccinated individuals is higher in the >65-year-old group. At the same time, when comparing the total number of vaccinated and unvaccinated hospitalised patients with laboratory-confirmed influenza in supplementary figure S2, the former was approximately four times lower on average. Therefore, the number of vaccinated individuals without hospitalisation records could be substantially higher, further reducing the OR of vaccinated *versus* unvaccinated individuals and potentially explaining why this ratio may exceed 1 in some instances (figures 3 and 4).

The mean prevalence of influenza virus infection in unvaccinated patients (figure 3a) was significantly higher than in the vaccinated group for both IAV and IBV, highlighting the effectiveness of vaccination as previously reported [18, 19]. However, when the analysis was performed by discriminating different influenza viruses, vaccination was highly effective in preventing IAV H1N1 and IBV infections and less effective against H3N2. These results corroborate the findings of other studies about vaccination effectiveness against H3N2 [20] and could cause concern for continued antigenic epitopes changes [21], waning immunity and haemagglutinin glycosylation, among others [22, 23]. Indeed, the wide evolutionary patterns of the H3N2 subtype, with different clades co-circulating at the same time, make it hard to select the best strain for the influenza vaccine, jeopardising a good match among vaccine strain and wild strain, thus reducing the effectiveness of the influenza vaccine against this specific subtype.

Influenza vaccination offers significant protection against mortality for up to 12 months post-infection (figure 4). This was particularly evident in individuals >65 years old, especially against H1N1 and H3N2 IAV infections, with less pronounced effects observed for IBV. However, the analysis based on the TriNetX dataset demonstrated a decline in vaccine effectiveness in terms of preventing death over time, which was notably more pronounced for H3N2; thus, annual vaccination, with optimal effectiveness observed when administered shortly before the influenza season, is an important current practice for reducing in-hospital influenza-related death. Additionally, vaccination correlates with a reduction in the incidence of co-infection with secondary pathogens associated with pneumonia, a leading cause of mortality among influenza-infected patients [11]. In the case of IBV, the data presented may not indicate

any apparent influenza vaccination protection from death. However, this result has to be interpreted with caution and taking other facts into consideration. IBV infections were associated with a lower mortality risk compared with IAV infections (figure 4b, c) in all the pre-existing conditions analysed using the TriNetX dataset (figure 5a). However, further studies are required to better understand this issue, such as understanding the patient's immune status and the comorbidities of hospitalised IBV-infected patients. In fact, some studies performed in certain countries showed similar or higher mortality rates for IBV compared to IAV [24–27]. Therefore, maintaining vaccination efforts is both necessary and beneficial.

Patients with certain underlying conditions are more susceptible to severe influenza disease [11]. Influenza vaccination was effective in reducing the mortality risk for all influenza virus infections despite the patient's comorbidities; for some comorbidities, vaccination resulted in no significant difference in the ORs compared to those for vaccinated patients without those comorbidities (figure 5a). The analysis presented here indicates that vaccination is particularly effective against mortality associated with IAV infections, and specifically against the H3N2 virus (figure 5b).

Our literature-based meta-analysis comprised patients from diverse countries, each with distinct healthcare systems and vaccination coverage and different life expectancies. It included patient data for 2003–2023, which allowed for a robust analysis. However, during this period, vaccines evolved such that in 2009, H1N1 strains used in the vaccine formulation were substituted by H1N1pdm09 strain [28], which may have contributed to differences before and after that event. Similarly, tetravalent vaccine (which added the second lineage of IBV) was not introduced until 2013–2014 [28]. Also, the COVID-19 pandemic and the consecutive lockdowns worldwide might have influenced the spread of influenza viruses. For instance, according to the Global Initiative on Sharing All Influenza Data [29] and FluNet [30], there has been no confirmed detection of the naturally occurring IBV/Yamagata virus since March 2020 [30]. The adjuvanted and the high-dose influenza vaccines had different speeds of implementation in different countries, which also alters elements of vaccination effectiveness in certain countries compared to others.

Overall, the results presented here delineate variations in infection rates among influenza-diagnosed patients across the most prevalent influenza viruses of clinical relevance, alongside evaluating the effect of vaccination and ageing within this cohort. The present study underscores the effectiveness of vaccination, while also assessing its effectiveness against each virus type across various demographic groups. By elucidating these factors, our findings offer insights that can inform healthcare policies and interventions, ultimately contributing to improved influenza management strategies and vaccination campaigns worldwide.

Influenza vaccination offers significant mortality protection, especially for high-risk groups such as individuals >65 years old, demonstrating notable effectiveness against H1N1 and H3N2 IAV infections. Further investigations are warranted to elucidate synergistic factors contributing to influenza severity, as well as to delineate precise risk factors and stratify immunological age differences within the >65-years-old group. These efforts could enhance the effectiveness of interventions aimed at reducing influenza-related mortality burden, beyond infection protection.

Study limitations

The published literature dataset was collected from articles published only in the PubMed database. For further studies, including other databases might result in a more precise analysis. The study is based on RT-PCR-confirmed influenza cases. The total number of asymptomatic and non-PCR-diagnosed cases is not considered in our analysis. This may affect some analyses, *e.g.* the prevalence study. It is known that IBV is commonly less severe than IAV subtypes in adults. For this reason, the number of asymptomatic patients may vary for each influenza type or subtype.

In the analysis of influenza-related mortality, the outcome "deceased" cannot be set as a direct consequence of viral infection, especially when considering the time from influenza diagnosis. Therefore, patients >65 years old or with comorbidities could be more affected by factors that could or could not be related to viral infection.

Finally, we used two different databases with common data. However, a comparison of the metadata included in each database shows variations because some published studies focused on specific patient data. For this reason, although the databases match in most aspects, the results from each database may present some differences when comparing ages or timespan.

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