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Challenges in estimating influenza vaccine effectiveness

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Abstract

Introduction: Influenza vaccination is regarded as the most effective way to prevent influenza infection. Due to the rapid genetic changes that influenza viruses undergo, seasonal influenza vaccines must be reformulated and re-administered annually necessitating the evaluation of influenza vaccine effectiveness (VE) each year. The estimation of influenza VE presents numerous challenges.

Areas Covered: This review aims to identify, discuss, and, where possible, offer suggestions for dealing with the following challenges in estimating influenza VE: different outcomes of interest against which VE is estimated, study designs used to assess VE, sources of bias and confounding, repeat vaccination, waning immunity, population level effects of vaccination, and VE in at-risk populations.

Expert Opinion: The estimation of influenza VE has improved with surveillance networks, better understanding of sources of bias and confounding, and the implementation of advanced statistical methods. Future research should focus on better estimates of the indirect effects of vaccination, the biological effects of vaccination, and how the vaccine interacts with the immune system. Specifically, little is known about how influenza vaccination impacts an individual's infectiousness, how the vaccine wanes over time, and the impact of repeated vaccination.

Keywords

influenza; vaccination; effectiveness; challenges; test-negative; case-control; cohort; bias; waning; confounding

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DECLARATION OF INTEREST

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1 Introduction

Each year seasonal influenza epidemics cause millions of influenza virus infections worldwide (1) resulting in millions of dollars spent on vaccination programs and treatments of patients. While most individuals who become infected with influenza have mild symptoms and recover without sequelae, some individuals can develop severe illness, particularly those with high risk conditions (e.g., the elderly, pregnant women, and young children) (2–5). An estimated 3 to 5 million cases of severe illness and 300,000 to 650,000 deaths are caused annually by seasonal influenza epidemics worldwide according to the World Health Organization (WHO) (1, 6). In the United States, an estimated 140,000 to 710,000 influenza-related hospitalizations and 12,000 to 56,000 influenza-related deaths occur each year (7). In the 2014–2015 influenza season, a particularly severe influenza season, an estimated 40 million influenza illnesses, 19 million influenza-associated medical visits, and 970,000 influenza-associated hospitalizations occurred in the United States (8). During the 2017–2018 influenza season, there were an estimated 80,000 deaths from influenza in the United States, alone (9).

Influenza poses a significant global public health burden and vaccination is the most effective way to reduce this burden. Due to the rapid genetic changes that influenza viruses undergo, seasonal influenza vaccines must be reformulated and re-administered annually to match circulating strains. Thus, vaccine effectiveness (VE), i.e., the vaccine-induced reduction in the risk of influenza compared to unvaccinated, must be estimated each season to quantify vaccine performance. Influenza VE varies from season to season and by individual factors (e.g., age and health status). Even in years when influenza VE is modest, vaccination can drastically lower the disease burden. For example, VE estimates from the 2015–2016 influenza season in the United States ranged from 24% to 57% (depending on age group), resulting in an estimated 5.1 million influenza cases, 2.5 million influenza-associated medical visits, and 71,000 hospitalizations that were averted by vaccination (14). The ability of the influenza vaccine to avert many cases even with suboptimal vaccine effectiveness is due to influenza's relatively low basic reproductive number (R_0). Mathematical modelers have estimated that the R_0 of influenza varies from 1.4 – 4 (15), with most estimates in the range of 1.5 – 2 (16). In other words, the average case of influenza living in a population that is 100% susceptible is capable of transmitting the infection to 1.4 – 4 persons.

When evaluating vaccine performance, the aim is to obtain an estimate that represents the causal effect of the vaccination on risk of influenza. Vaccine efficacy refers to the relative reduction in the risk of disease in a vaccinated person through direct effects of the vaccine assessed through a randomized clinical trial and is estimated as one minus the relative risk of disease in vaccinated individuals compared to unvaccinated individuals (10). Vaccine effectiveness refers to the impact of a vaccine assessed using observational studies. Because VE is an estimate of a causal effect from observational studies, any estimate of VE should be adjusted for potential confounding variables, i.e., variables associated with both vaccination and risk of influenza (11). Therefore, VE is defined as one minus the adjusted relative risk of disease in vaccinated individuals versus unvaccinated individuals, and should not be estimated by crude, unadjusted relative risks in observational studies. In an outbreak

situation, as in a seasonal influenza epidemic, relative risk can be expressed as the ratio of attack rates in the vaccinated and unvaccinated (12). While the definition of VE in terms of the ratio of cumulative attack rates in vaccinated and unvaccinated person is widely accepted, some studies (13) define VE as one minus the corresponding adjusted odds ratio or the adjusted ratio of the hazard rates (the instantaneous risk of infection). While attack rates are easier to interpret, hazard rates are more meaningful from the statistical perspective. Throughout this review any mention of an estimate of VE, such as one minus the relative risk, refers to an estimate adjusted for confounders.

The estimation of VE presents numerous challenges. This review focuses on challenges of estimating vaccine effectiveness, which by definition is obtained from observational studies, whereas vaccine efficacy is obtained from randomized clinical trials. Therefore, we considered randomized clinical trials outside the scope of this review and do not discuss them here. This review aims to identify, discuss, and, where possible, offer suggestions for dealing with the challenges in estimating seasonal influenza VE. Specifically, we focus on different outcomes of interest against which VE is estimated, study designs used to assess VE, sources of bias and confounding, repeat vaccination, waning immunity, population level effects of vaccination, and VE in at-risk populations. Estimation of VE during an influenza pandemic involves additional challenges that will not be addressed in this review.

2 Outcome of interest

When estimating influenza VE, the outcome against which the vaccine is expected to protect must be specified because VE may differ depending on the outcome against which it is estimated. For example VE against any manifestation of influenza virus infection may be different from VE against influenza-related hospitalization, which may be different from the VE against transmission of the virus. Several studies have shown that under certain conditions VE estimates differ against symptomatic influenza and medically-attended influenza (17–20). In this section we discuss different outcomes against which VE may be estimated (Table 1) and the challenges associated with each.

2.1 Asymptomatic influenza

Symptoms of influenza illness are usually referred to as acute respiratory illness (ARI). Not all individuals infected with influenza develop an ARI, some remain asymptomatic. However, asymptomatic individuals may still be infectious (21), but less so than symptomatic individuals because they do not shed as much virus (22). Estimates of the proportion of asymptomatic individuals in a given influenza epidemic vary widely with studies estimating that the proportion of individuals infected with influenza who develop symptoms are 23% (23), 67% (21), or 84% (24). The role of asymptomatic individuals is important to consider when assessing disease dynamics and estimating intervention effectiveness, such as vaccination. While asymptomatic individuals are less infectious, they may have a similar or greater role in perpetuating an outbreak than their symptomatic counterparts because they may make more contacts, as they do not realize they are infected (e.g., they will not stay home from work or school). Current estimates of VE fail to capture asymptomatic individuals as routine surveillance focuses on symptomatic cases (usually,

individuals who seek medical care). Studies designed to estimate VE against asymptomatic influenza are difficult and expensive as these studies require frequent testing of study participants to capture asymptomatic cases while they are shedding influenza virus.

2.2 Symptomatic influenza

Symptomatic influenza is defined as influenza virus infection that results in ARI. Observational studies designed to estimate VE against symptomatic influenza, namely active surveillance cohort studies, are expensive and logistically complicated because they involve study personnel actively testing study participants when they develop influenza-like symptoms. Thus, these types of studies are not carried out as frequently as studies against other outcomes, such as test-negative studies designed to estimate VE against medically-attended influenza. Studies designed to capture any symptomatic individual allow for the estimation of VE against symptomatic influenza of any severity regardless of whether an individual seeks medical care (13, 25, 26). Several cohort studies have been set up to estimate VE against symptomatic influenza annually (13, 25, 27). Evaluating VE against symptomatic influenza is important as influenza patients who do not seek medical care add to the burden of disease. Additionally, the general public assumes reported VE in studies designed to estimate VE against medically-attended influenza is VE against symptomatic influenza.

2.3 Medically-attended influenza

Outpatient medically-attended influenza is the most commonly used outcome in influenza VE studies, particularly with the growing popularity of the test-negative (TN) design and its incorporation into influenza surveillance networks (28–30). In this subsection we discuss the use of outpatient medically-attended influenza as an outcome of interest. In the following subsection, we discuss inpatient medically-attended influenza. Medically-attended influenza is defined as influenza virus infection resulting in an ARI for which a person seeks medical care. In addition to the TN design, passive surveillance cohort and traditional case-control studies estimate VE against medically-attended influenza because they define cases as individuals who seek medical care for ARI and test positive for influenza virus infection. In most of these studies, the outcome of interest is influenza illness resulting in an outpatient visit. Some studies assess VE against more severe outcomes, such as influenza-related hospitalization, admission to an ICU, or death.

While it is convenient to conduct VE studies in medical care settings, individuals who seek medical care and get tested may not represent the entire population resulting in selection bias, if the goal is to really measure the effectiveness against symptomatic influenza. Coleman *et al.* compared TN-based VE estimates during the 2004–2005 to 2006–2007 influenza seasons estimated either when attempts were made by study personnel to test all persons presenting with ARI or persons were only tested when a clinician ordered an influenza test. They found that VE estimates against medically-attended influenza were 5–33% lower in the clinician testing group compared to the entire population (31). Care must be taken when communicating VE estimates against medically-attended influenza because the true VE against medically-attended and symptomatic influenza differ if vaccination influences the probability of seeking medical care for influenza (19).

2.4 Hospitalizations/Severe Influenza Illness

Influenza vaccines have been shown to be effective against hospitalizations (32–34) and severe or fatal outcomes (35, 36). Arriola *et al.* found that influenza vaccination attenuated adverse outcomes in hospitalized adults in all age categories (37). Few studies have compared the effectiveness of the influenza vaccine for different outcomes. Castilla *et al.* compared VE estimates against outpatient, inpatient, and severe or fatal cases of influenza during the 2010–2011 influenza season. They found that VE estimates differed by outcome, with VE highest against severe or fatal cases (89%) and lowest against hospitalizations (60%) (VE was 75% against outpatient cases) (35). Remschmidt *et al.* found that VE estimates were higher in hospitalized cases (2010–11 season: 82%; 2011–12 season: 63%; 2012–13 season: 66%) compared to non-hospitalized cases (2010–11 season: 72%; 2011–12 season: 32%; 2012–13 season: 57%) for influenza A in adults 60 years old or older in three influenza seasons (34). A 2016 systematic review found no difference in VE estimates in inpatient and outpatient settings (38). These studies highlight that is important to communicate the outcome against which VE is estimated, as it may vary substantially depending on the outcome. One explanation for the difference in VE estimates between inpatient and outpatient settings is some patients may no longer be infected (and therefore become false negative) by the time they are hospitalized or develop a complication, leading to biased estimates of VE.

3 Study Design

Vaccine effectiveness refers to the impact of vaccination on risk of influenza estimated using observational studies. In this section we discuss the different observational study designs used to estimate VE and the advantages and disadvantages of each (Table 2).

3.1 Identifying cases

Accurate estimation of influenza VE relies on the ability to identify cases; however, the identification of influenza cases is difficult because it is not easy to find all or most influenza patients in each community, as symptoms are frequently mild, and many patients do not seek medical care to alleviate them. Additionally, symptoms of influenza are non-specific; hence many patients who develop ARI are not infected with the influenza virus. Finally, laboratory tests are required to confirm influenza virus infection, and these tests are not 100% sensitive and specific.

3.2 Cohort studies

In cohort studies used to assess influenza VE, a cohort of members of the study population are identified prior to the influenza season and followed throughout the study period (usually the entire season). There are two main types of influenza cohort studies: active surveillance cohort and passive surveillance cohort. In active surveillance cohort studies, cases are defined as individuals in the cohort who reported an ARI and test positive for influenza. Study participants report each occurrence of ARI symptoms to study personnel and are then tested for influenza virus infection regardless of whether the participant seeks medical care for their symptoms. In passive surveillance cohort studies, cases are only selected from individuals in the cohort who seek medical care for ARI and subsequently test positive for

influenza virus infection. Non-cases in both study designs are defined as all study participants who are not considered cases. Active surveillance cohort studies aim to test everyone who develops ARI for influenza and thus can capture cases with influenza illness of any severity, not just those that necessitate seeking medical care. The prospective nature of cohort studies allows for the collection of information on timing of infection and vaccination. Cohort studies also provide a means of determining influenza VE in areas where there are no surveillance systems to routinely collect reports of influenza illness, such as Japan (39).

VE is usually estimated in cohort studies as one minus the relative risk (40–42), where relative risk is estimated by comparing the cumulative incidence of the outcome (e.g., influenza illness) among the vaccinated to that among the unvaccinated, adjusting for confounders (43). Statistical methods used in the estimation of VE include logistic regression, Poisson multi-level modelling, the screening method (44), and the Cox proportional hazards model (45) (as one minus the hazard rate ratio (13, 27)). However, caution should be used when comparing results from different studies using different statistical methods as estimates have been shown to vary by methodology (44).

Another complication of VE cohort studies is that vaccination status may change during the study period. Estimating relative risk using attack rates makes the implicit assumption that exposure (e.g., vaccination) is the same for each person in the exposed group. However, an individual vaccinated at the beginning of the study has a different exposure than someone vaccinated in the middle of the study period. VE can be estimated by identifying events based on vaccinated vs. unvaccinated person-time and calculating rate ratios or hazard ratios.

A third type of cohort study, a monitored household study, is a special type of active surveillance cohort study in which entire households are enrolled in the study instead of unrelated individuals. A monitored household study design has recently been adopted to assess influenza VE (13, 46, 47) because transmission of influenza within the household has been shown to play an important role in influenza disease dynamics (48, 49). During the influenza season, there are potentially two different types of influenza exposures: exposure to other infected household members and exposure to infected individuals in the larger community. Unlike active and passive surveillance cohort studies, monitored household studies allow for the separate estimation of VE against household-acquired and community-acquired influenza virus infection (13), which may be different (50).

3.3 Case-control studies

Traditional case-control studies have historically been used to estimate influenza VE when randomized clinical trials were not feasible (51). Within traditional case-control studies, cases are defined as members of the study population who seek medical care for an ARI and test positive for influenza virus infection. Controls are randomly selected people who did not develop an ARI during the study period from the same population. VE is defined as one minus the adjusted odds ratio comparing the odds of vaccination in cases and controls, adjusted for confounders (41, 52, 53). Traditional case-control studies can provide useful information about vaccine performance at the population level but are subject to selection bias because cases are selected only from people infected with influenza virus who sought

medical care, while controls are randomly selected from the entire population. To minimize bias and confounding, it is recommended that case-control studies be carefully designed and implemented, including using specific endpoints, starting the study soon after vaccine introduction, selecting the control population carefully, and matching cases and controls only on key covariates thought to be associated with vaccination and the outcome (54). More recently, a newer case-control study design, the TN design, has been used to estimate influenza VE in an attempt to reduce the bias introduced in a traditional case-control study. Since first being used to assess influenza VE in 2005 (53), TN studies have become the most popular design for assessing annual influenza VE. The TN design was originally used to estimate the effectiveness of the pneumococcal vaccine (55). In addition to influenza, this study design has also been recently used to evaluate vaccines against rotavirus (56–59), cholera (60, 61), and meningococcus (62). Within a TN study, cases are selected from individuals who seek medical care for ARI and test positive for influenza virus infection, while controls are individuals who seek care for ARI and test negative for influenza virus infection. The TN design is attractive because it can be easily incorporated into existing surveillance systems and attempts to control for confounding due to propensity to seek medical care because cases and controls are both selected from individuals who seek medical care for ARI (63).

An important assumption underlying the validity of the TN design is the exposure (e.g., influenza vaccine) has no effect on the probability of developing the control outcome (e.g., a non-influenza ARI). Studies designed to test this assumption reached conflicting results. In a randomized clinical trial, Cowling *et al.* found that vaccinees had a significantly increased risk of developing a non-influenza ARI compared to non-vaccinees (64). Other studies found no increased risk of developing a non-influenza ARI in vaccinees compared to non-vaccinees (40, 65).

Concern has been raised about the validity of TN-based VE estimates, particularly due to the study design's growing popularity. Previous work has demonstrated the validity of TN-based estimates of VE if vaccination does not affect the probability of developing non-influenza ARI in outpatient settings (40, 52, 66–68), in inpatient settings (69), and when the influenza test has imperfect sensitivity and specificity (41). However, selection bias may be introduced into estimates of VE obtained from TN studies because cases and controls are selected only from persons who seek medical care (70). Hence, VE estimates from TN studies may not accurately reflect the VE in the entire population of cases and controls.

Recent work has raised concern about clinician bias in TN studies. A recent study by Thompson *et al.* in which an administrative database was searched for codes listing acute respiratory or febrile illness found that only 5% of the 19,450 of the cases with these diagnostic codes were tested for influenza (71). Bias could result if vaccinated cases were less likely to be tested than unvaccinated cases (72). This could lower the proportion of laboratory confirmed cases that were caused by the influenza virus among vaccinees and lead to falsely high measurement of effectiveness. These findings suggest that clinicians may subjectively select which patients get tested for influenza and highlights the importance of developing standard criteria for who gets tested for influenza virus infection. To avoid

selection bias, all study participants with the pre-specified symptoms of influenza-like illness or ARI should be tested for influenza virus infection.

4 Sources of bias and confounding

Bias may be present in observational studies for the estimation of influenza VE because differences in the risk of outcomes (e.g., infection) may exist that are caused by factors other than vaccination status. Depending on the study design, this bias may be a result of differences in the selection of cases and controls, such as a) differing health-care seeking behavior between vaccinees and non-vaccinees or the effect of vaccination on the probability of developing non-influenza ARI; b) confounding, such as the effect of health status or frailty on both the probability of vaccination and the probability of influenza ARI; or c) misclassification of influenza virus infection and/or vaccination status. In the following section we discuss three types of bias: selection bias, confounding, and information bias. However, the sources of bias discussed below are not exhaustive and additional bias in influenza VE studies may result from sparse data, missing data, and volunteer bias, among others.

4.1 Selection Bias

Selection bias occurs when the association between vaccination and risk of influenza in the study population differs from the association in the full population (70, 73). In influenza VE studies, selection bias may result from differences in vaccinees and non-vaccinees (74). For example, vaccination may have an effect on the probability that an individual is selected as a case or non-case/control. If vaccination reduces symptom severity of influenza illness (35, 75, 76), a vaccinated influenza patient will be less likely to seek medical care for ARI than their unvaccinated counterpart, which may impact their probability of being included in the study. In a passive surveillance cohort or traditional case-control study, where cases are selected from individuals who seek medical care, and controls are not, vaccinated individuals would be less likely to be considered a case than unvaccinated individuals. A TN study attempts to control for this by selecting both cases and controls from persons seeking medical care for an ARI (77). Studies have found that TN-based VE estimates against medically-attended influenza are not affected by selection bias if vaccination affects the probability of seeking care in the absence of other sources of bias. However, TN-based VE estimates against symptomatic influenza may overestimate the true VE (18–20). An active surveillance cohort study removes this source of bias because cases and non-cases can be identified regardless of whether a participant seeks medical care for an ARI.

Selection bias may also occur if vaccination modifies the probability of developing non-influenza ARI because of virus interference. As a result, in TN studies, the probability of being considered a control depends on vaccination status because individuals who seek medical care for non-influenza ARI are used as controls. However, two simulation studies have shown that if the influenza vaccine increases the risk of non-influenza ARI, the bias of VE estimates will only be severe in extreme cases (19, 78).

4.2 Confounding

Confounding results from an outcome and an exposure sharing a common cause (73) and is known to bias influenza VE estimates. Jackson *et al.* estimated VE against all-cause mortality in seniors using the adjusted hazard rate ratio at three time points: prior to the influenza season, in the autumn, and in the winter. The study found that the vaccine association differed with time providing evidence that the observed effect of vaccination (particularly prior to the season when no virus is circulating) is all, or partially, due to confounding (79). Common sources of confounding adjusted for in influenza VE studies are age, calendar time, and health status (80).

Age is a well-established confounder of influenza VE estimates because vaccine coverage and the risk of influenza virus infection can both vary considerably with age (80). However, there is no unified approach for how to adjust for age when estimating VE. For example, age has been included (typically in logistic regression) as a categorical variable, a linear term, a cubic spline, and a quadratic term (80). Different methods of adjustment for confounders can lead to differing estimates of VE. Thus, further research should focus on comparisons of methods to adjust for confounding to determine best practices.

Health status is a known confounder in influenza VE studies, particularly in studies among the elderly because a person's health status may be associated with both their probability of being vaccinated and becoming infected with influenza virus (81, 82). A frail person, or person with low health status, may be more likely to be vaccinated because they are considered at higher risk for influenza virus infection. This phenomenon would result in less unvaccinated cases, biasing VE estimates downwards. Conversely, healthy persons may be more likely to be vaccinated to preserve their good health (81, 83) resulting in fewer vaccinated cases, which bias VE estimates upwards. Frailty has been shown to confound estimates of VE in simulation studies (18–20) and in studies in elderly populations (32, 84).

Another confounder of influenza VE against medically-attended influenza is a person's health awareness (i.e., their behaviors and attitudes towards vaccination and seeking medical care) (19). For example, consider a person with high health awareness (e.g., has good hygiene practices and visits the doctor regularly). This person may be more likely to be vaccinated and also more likely to seek medical care if they develop an ARI. In studies that define cases as individuals who seek medical care and test positive for influenza virus infection, confounding due to health awareness may cause higher numbers of vaccinated cases compared to controls, biasing VE estimates downwards. Health awareness is an unobservable variable, making adjustment difficult (77). However, using imperfect proxy variables have been shown to reduce bias (85, 86).

4.3 Information bias

Information bias may occur in influenza VE studies as a result of incorrectly determining influenza virus infection status, vaccination status, or individual covariate values. In this section we discuss information bias in influenza VE studies resulting from misclassification of influenza virus infection status and vaccination status.

The current gold standard for detecting influenza virus infection in studies to estimate influenza VE is by reverse transcriptase polymerase chain reaction (RT-PCR) tests, although other methods such as rapid influenza diagnostic tests (RIDT) or viral culture are used. RT-PCR and viral culture have high sensitivity and specificity (87), while RIDTs have a sensitivity of only 50–70%, resulting in a higher proportion of false negative results, and a specificity of 90–95% (88). Because influenza tests are not 100% sensitive or specific, misclassification of influenza virus infection status can occur. Jackson *et al.* used simulations to investigate the effect of imperfect test sensitivity and specificity on VE estimates from TN, traditional case-control, and cohort studies. The study found that all three study designs underestimated VE in the presence of misclassification of infection status. However, the amount of bias was trivial when using RT-PCR due to the test's high sensitivity and specificity (41). A more recent study found that VE estimates from active surveillance cohort, passive surveillance cohort, TN, and traditional case-control studies were robust to imperfect test sensitivity, but more susceptible to bias when the test had imperfect specificity in the presence of other sources of bias. For example, when ARI patients with high health awareness have a higher probability of seeking medical care compared to patients with low health awareness TN-based VE estimates were unbiased when the influenza test was 90% sensitive and 100% specific, but suffered from bias (absolute value of bias = 0.12; true VE = 0.46) when the influenza test was 100% sensitive and 90% specific (19). Thus, caution should be used when interpreting estimates of VE from studies that confirm influenza virus infection with tests other than RT-PCR, and, when possible, RT-PCR should be used to confirm influenza virus infection.

An additional source of information bias may occur from misclassification of vaccination status, which may result from poor patient recall, the inability to verify vaccination status from medical records, or incomplete or incorrect medical records (80). Numerous major influenza surveillance networks rely on self-reporting to determine influenza vaccination status (28–30). Self-reporting of vaccination status has been found to result in false positives, i.e., unvaccinated individuals reporting that they received the influenza vaccine. Studies of patient recall of vaccination status have estimated sensitivity around 95% and specificity around 90%. Even with high values of recall sensitivity and specificity, estimates of VE may be overestimated (80). A simulation study found that the misclassification of vaccination status can lead to meaningful bias of VE estimates, and in extreme cases, can approach a relative bias of 50% (true VE = 0.5 and estimated VE = 0.26) (89). Caution should be exercised when interpreting influenza VE estimates from studies that use self-reported vaccination status, and, when possible, medical records should be used to verify vaccination status.

5 Vaccination

Many factors related to influenza vaccines may influence estimates of VE. In this section we discuss types of vaccines, mechanisms of vaccine protection, repeat vaccination, waning immunity from vaccination, and the population-level impacts of influenza vaccines. We discuss each topic's impact on estimates of VE.

5.1 Types of vaccines

There are many different types of influenza vaccines being used around the world. The original vaccines were killed whole viruses but because of concerns about reaction rates, today the vast majority of vaccines, and all vaccines used in countries like the United States, are split virus or subvirion inactivated vaccines or live attenuated vaccines (90). The inactivated vaccines have standardized quantities of hemagglutinin (usually 15 micrograms per influenza strain) and contain three (trivalent) or four (quadrivalent) influenza strains (90, 91). The trivalent vaccines contain representative strains of influenza A/H1N1 and A/H3N2 and one influenza B lineage (either B Yamagata or B Victoria). Quadrivalent vaccines contain representative strains of both type B lineages. The vaccines usually contain other antigens such as the neuraminidase of the given strains, however, they are not standardized to have a specific quantity of those antigens in contrast to the specific quantity of hemagglutinin (91). In addition, there are high dose vaccines (i.e., 45–60 micrograms per antigen per strain licensed for use for persons ≥ 65 years) and adjuvanted vaccines. Almost all inactivated vaccines are administered intramuscularly although, an intradermal vaccine has been available (90, 91).

The great majority of influenza vaccine viruses are replicated in the allantoic cavities of embryonated hens' eggs and thus use "egg adapted strains", which may change the immune response compared to that induced by non-egg adapted strains (92). However, the actual impact of egg adaptation on effectiveness is controversial (93). There are two inactivated vaccines that are not made from egg adapted strains and are not produced in embryonated chicken eggs (91). One is produced in cell culture and the other is produced by inserting the gene for the hemagglutinin into a baculovirus which then infects insect cells and produces hemagglutinin for the vaccine. Live attenuated influenza vaccines (LAIV) are produced by inducing reassortment of wild strain influenza viruses with cold adapted live attenuated influenza viruses (94). The hemagglutinin and neuraminidase are derived from the wild strain whereas the remaining six genes are derived from the cold adapted attenuated strains. LAIV is administered intranasally and the LAIV used in the United States is a quadrivalent product.

With the myriad of vaccine products in use to protect against influenza virus infection, VE estimates often vary by study depending on the vaccine(s) used by the study participants. Some studies identify which vaccine they are estimating VE against, such as the trivalent inactivated influenza vaccine, but studies rarely specify the manufacturer (80). This poses a problem with the interpretation of the effectiveness of all influenza vaccines combined as a single measure, as the protection of one vaccine product may not match the protection of a different product. Although individual study participants are likely to receive only a single type of influenza vaccine, the group of study participants as a whole may be vaccinated with a number of different vaccine types from numerous manufacturers, so VE estimates are a composite estimate of numerous vaccines.

5.2 Mechanism of protection

There are two possible mechanisms by which vaccines confer protection. The first is the "all-or-none" model where a proportion of vaccinated individuals, ρ , are conferred complete

immunity (100% protection) from infection, while the remaining proportion, $1-\rho$, are fully susceptible to infection. The second is the “leaky” model where the vaccine reduces the probability of infection by a fraction, ρ , in a given individual but does not confer complete immunity in that individual (42, 95).

Under randomized vaccine uptake and the “all-or-none” model, the odds ratio can recover the proportion of individuals protected by vaccination (42, 96). On the contrary, under the “leaky” model the odds ratio does not accurately recover the reduction in probability of infection conferred by the vaccine (97). In practice, it is very difficult to determine which of the two mechanisms is more appropriate to model the effect of the influenza vaccines. It is very possible that the true effect is a mixture of both mechanisms.

5.3 Repeat vaccination

Due to antigenic drift, influenza vaccines are updated frequently to maintain genetic similarity between the vaccine strains and the projected circulating strains. This necessitates the evaluation of influenza vaccines annually; however, an important confounder not usually accounted for in the evaluation of influenza VE is influenza vaccination in previous seasons (77, 98).

Public health organizations have recommended annual vaccination to high risk individuals since 1960 (99), and have more recently extended the recommendation to all individuals over the age of 6 months in Ontario and the United States (100). As more people are annually immunized, repeated influenza vaccination has received growing interest (101).

Vaccination in the previous season(s) may affect VE in the current season in different ways. Past vaccination may still afford some protection in the current season if the circulating strain has not drifted far from the prior vaccine strain (13, 102). On the other hand, those who were not vaccinated in the previous season(s) have had more chances to become infected and this past infection may increase a person’s immune response in the current season. It is difficult to find information on infections in past seasons. Some modelling approaches have been developed to infer past infections (103), but these do not yet have the capability to distinguish past infection from past vaccination. Furthermore, multiple past vaccinations may focus the immune response toward epitopes present in prior vaccines rather than on the new epitopes present in the most recent year’s vaccines. Thus, the new vaccine theoretically may not induce adequate immunity against the drifted epitopes, critical to protecting against new drifted strains.

Historically, studies have reported inconsistent findings regarding whether repeated vaccination is detrimental to protection against influenza virus infection (104–106). Several recent studies have found lower VE among individuals who were vaccinated in the current season and the prior season compared to individuals only vaccinated in the current season (26, 107, 108). However, a 2017 systematic review and meta-analysis found no evidence that prior season vaccination negatively impacts current season VE (101).

Evidence suggests that the effects of repeated vaccination may vary by influenza type and subtype. Negative effects of repeated vaccination have been found to be more associated with influenza A/H3N2 compared to A/H1N1 and B (109–111).

In a recent paper, Foppa *et al.* conducted a theoretical assessment of the potential bias caused by vaccination history. The study found that failure to account for vaccination history caused nontrivial bias in VE estimates from TN studies, but full and accurate adjustment for prior vaccination history removed bias in the absence of misclassification of vaccination history. However, in practice determining a complete vaccination history is impractical, so the authors explored partial adjustment (accounting for one or two prior seasons) and found that it reduced the bias of VE estimates (112). Numerous statistical methods have been used, such as propensity scores to adjust for the likelihood of being vaccinated (113), logistic regression with an interaction term for vaccination history (111), and a Cox proportional hazards model (13) to attempt to adjust for any bias caused by repeated vaccination. Without a clear understanding of the impact that repeated vaccination has on VE estimates, employing the most appropriate statistical method to estimate VE remains difficult, especially in the presence of misclassification of vaccination status, which has been shown to compromise efforts to adjust for vaccination history (112).

5.4 Waning of the vaccine effect

Determining if protection from vaccination wanes over time during the influenza season in which a person was vaccinated has received a lot of attention in recent years. Several studies conducted in Europe during the 2011–2012 influenza season found that vaccinated individuals tended to present with influenza virus infection later in the season, suggesting some degree of waning immunity from vaccination (114–117). Since the 2011–2012 season, a season characterized by late onset, studies conducted in Australia (118, 119), the United Kingdom (120), Europe (121), and the United States (122–124) have found conflicting results regarding intraseason waning. Ferdinands *et al.* pooled data from the 2011–2012 through 2014–2015 influenza seasons and observed decreasing influenza vaccine protection as time since vaccination increased (122). However, this study only showed an association between VE and time since vaccination, not causation. Some argue that decreases in VE may be due to artifact rather than waning. If influenza vaccination provides “leaky” protection, that is, lowers the probability of infection rather than render complete immunity, the rates of infection between vaccinated and unvaccinated individuals will become similar with increasing time since vaccination. This would result in a decrease in the VE estimate that is not the product of waning immunity (125, 126). An alternative explanation of intraseason waning is antigenic drift in circulating viruses away from the viruses included in the vaccine (124).

If intraseason waning of protection from the influenza vaccine is a true biological phenomenon, it is important to consider the impact of waning immunity on estimates of VE. In the presence of waning, we would expect estimates of VE to decrease during the season. In a randomized controlled trial, Petrie *et al.* estimated time-varying VE in healthy adults for both the inactivated influenza vaccine (IIV) and the LAIV. Their results showed that the IIV waned significantly during the influenza season but was still efficacious by the end of the

season. In contrast, the LAIV did not show evidence of waning, but was less efficacious (127). Radin *et al.* found consistent VE 0–180 days post vaccination, suggesting no intraseason waning, but found a decrease in VE 181–365 days post vaccination (128).

Determining whether influenza vaccine protection wanes over time is further complicated by the fact that influenza vaccines contain multiple influenza strains. A systematic review and meta-analysis found a significant decline in VE for A/H3N2 and B, but not for A/H1N1 (129), while Ferdinands *et al.* found decreasing VE with increasing time since vaccination for A/H3N2, A/H1N1, and B (122). Belongia *et al.* found that increased infection rates for A/H3N2 was associated with increasing time since vaccination among young children and older adults within a single influenza season (130).

An additional concern about the estimation of VE in the presence of waning is the timing of VE estimation. For example, if waning is present and VE is estimated at the end of the season, estimates will be lower than if VE was estimated at the beginning of the season. The estimation of waning effects of vaccines is difficult and requires special statistical methods, such as the smoothed case hazard rate ratio developed by Durham *et al.* for the investigation of waning immunity associated with cholera vaccines (131) and used by Petrie *et al.* to determine whether waning occurs with influenza vaccination (127). Additionally, the duration of vaccine-induced protection is important to consider when making policy decisions, such as when to begin vaccination programs or whether to recommend a second dose of the vaccine to individuals with a high risk of severe complications. However, current evidence is not definitive enough to disentangle potential waning from other causes of a decline in VE and a clearer understanding of the reasons for decreasing VE are needed (125).

5.5 Population-level impact of influenza vaccination

Vaccination not only reduces the probability that individuals who receive the vaccine will get infected, but also indirectly lowers the probability of transmission to both vaccinated and unvaccinated individuals because they are exposed to fewer infected persons. This phenomenon is called indirect protection or ‘herd immunity’ (132). The ordinary estimate of VE is based on comparing the risk of infection in a vaccinated and an unvaccinated person. Therefore, it is called ‘direct effectiveness’ or ‘individual effectiveness’ (95, 133). Health authorities and policy makers are often interested in the overall population-level effectiveness, i.e., the proportion of influenza cases averted by vaccination. The population vaccine effectiveness (also called ‘vaccine impact’) is defined as one minus the ratio of the observed attack rate in the partially vaccinated population and the expected attack rate in the same population if no vaccination took place. This estimate depends on both the direct and indirect effects of the vaccination program. However, it is very difficult to estimate the expected attack rate in the same, or a comparable, population without vaccination.

Cluster-randomized trials where some communities are vaccinated and others remain unvaccinated are unethical and difficult to carry out. Alternatively, it is possible to use modelling techniques to estimate the expected attack rate in the study population if no vaccination took place. Arinaminpathy *et al.* used a Bayesian stochastic model to estimate the population effectiveness of influenza vaccination in the United States. They found that

vaccination averted 20.8% (95% CI: 16.8, 24.3) to 47.5% (95% CI: 43.7, 50.8) of potential influenza virus infections depending on the season (134).

6 At-risk populations

The World Health Organization defines individuals at high risk of influenza virus infection, i.e., those who should be targeted for influenza vaccination, as pregnant women, children under the age of five years old, the elderly (persons over 65), and persons with comorbidities such as HIV/AIDS, chronic heart or lung disease, and asthma (135). Estimating VE in at-risk populations is difficult due to the heterogeneous nature of these populations (such as healthy seniors versus seniors with comorbidities), different vaccine recommendations compared to the general population (135), and access to at-risk individuals. For example, child participation in influenza VE studies requires the consent of a parent; therefore, selection bias may be present as children with parents in favor of vaccination may be more likely to participate.

6.1 Elderly

VE studies in the elderly face several challenges, namely confounding, the use of non-specific outcomes, and the receipt of different types of vaccines. Confounding is an established challenge in estimating VE in this group due to the healthy vaccine effect whereby healthy seniors are more likely to be vaccinated than frail seniors (81). Some confounding or bias can be removed using appropriate statistical methods. However, many observational studies of VE estimate VE in the elderly retrospectively using data from health care (136, 137) or research databases. These databases do not necessarily have information on confounders, such as functional status, severity of illness, living situation, or mobility (138), making appropriate adjustment for confounding difficult. Research has shown that seniors with higher mobility (i.e., who have access to a car or can walk to their healthcare provider's office (139)), live with people who can assist them (140), or have fewer functional limitations are more likely to be vaccinated (81). Furthermore, the use of non-specific outcomes, such as all-cause mortality (136), may be more susceptible to confounding than specific outcomes because individuals near the end of life may be less likely to be vaccinated (138).

The elderly shoulder a disproportionate burden of influenza annually because they have the highest risk and rates of hospitalization and severe outcomes. Additionally, influenza VE is lower in the elderly compared to working adults (141) which may be due to diminished immune response (142) or repeat vaccination (109, 143). As a result of decreased VE in this population, adjuvanted or high dose vaccines are available and some experts preferentially recommend such vaccines for the elderly (144). Thus, seniors may receive a different kind or dosage of vaccine compared to the majority of the population reducing the generalizability of overall VE estimates to the elderly population.

6.2 Pregnant women

The World Health Organization and other national public health agencies recommend annual receipt of an influenza vaccine for pregnant women (145). The rationale behind vaccinating

pregnant women is two-fold: 1) provide direct protection to the mother because they are at higher risk of developing severe complications following influenza virus infection (146), and 2) provide indirect protection to the infant through transplacental antibody transfer (145).

Unlike in the general population, estimating VE in pregnant women is complicated by the change in risk of influenza virus infection with gestational age, which may introduce bias into VE estimates if not appropriately accounted for (147). Time-varying Cox proportional hazard models (148) or nested case-control studies (149) have been used to reduce bias in VE estimates in this population. Additionally, it is well-known that there are differences in the health profiles of pregnant women who do and do not receive an influenza vaccine (148). Finally, the relatively small number of pregnant women who develop an influenza virus infection reduces the statistical power to detect the effectiveness of influenza vaccines (150) particularly against severe outcomes, such as hospitalizations, due to the even smaller sample sizes (135). To examine influenza VE against laboratory-confirmed influenza-associated-hospitalizations, the Pregnancy Influenza Vaccine Effectiveness Network (PREVENT), the largest observational study of influenza VE in pregnant women to date, identified pregnant women across 5 study sites in 4 countries between 2010 and 2016 (71). Using a retrospective TN study, PREVENT found that the VE against laboratory-confirmed influenza-associated-hospitalization was 40% (95% CI: 22–67%) after adjusting for site, season, season timing, and high-risk medical condition.

6.3 Children

Children have the highest rates of influenza virus infection and subsequent complications of any age group (135). Public health authorities recommend vaccination of young children to help prevent the transmission of influenza as their increased susceptibility is thought to drive epidemics (151–153). Unlike adults, it is recommended that children 6 months to 8 years of age being vaccinated for the first time get two doses of influenza vaccines at least 4 weeks apart (90). Additionally, the LAIV is recommended in this age group creating several challenges in VE estimation when there is a need to compare the effectiveness of LAIV versus IIV. Some studies of VE in children include children who have received only one dose of the vaccine, and thus may not be optimally protected by vaccination, but are considered “vaccinated”.

7 Conclusion

The challenges of estimating influenza VE are numerous and complex. Evidence continues to suggest that influenza vaccines are protective against infection; however, depending on the season, vaccine, and study, estimates of VE vary. This is partially due to bias and confounding, which may be mitigated by careful study design, outcome selection, and statistical method implementation. However, some of the variability in VE estimates is likely due to immunological response to vaccines over time. Future studies should focus on the change in immunological response to repeated vaccination, the potential waning of vaccine-induced immunity, and the genetic differences in egg-adapted vaccine strains compared to non-egg adapted strains to better understand how the true effectiveness of influenza vaccines varies over diverse immunological landscapes and time.

8 Expert Opinion

The estimation of influenza VE has improved with surveillance networks, better understanding of sources of bias and confounding, and the implementation of advanced statistical methods. The popularity of the TN design and its incorporation into surveillance networks provides annual estimates of influenza VE, helping to routinely assess vaccine performance. However, the TN study design only uses data from ARI patients who seek medical care and TN-based VE estimates are not generalizable to the entire population. While determining how vaccines protect against medically-attended influenza and other severe outcomes, such as hospitalization, is valuable, it is important to realize that medically-attended cases only make up a fraction of the annual burden of disease. Active surveillance cohort studies use data from all study participants with ARI regardless of their propensity to seek medical care, allowing the estimation of VE against symptomatic influenza and of the probabilities of seeking care among influenza and non-influenza ARI patients. In the future, investigators should plan and conduct concurrent TN and active surveillance studies in the same season and population to harness the advantages of both study designs. Concurrently conducting the two studies is expected to produce VE estimates with smaller bias than that of a TN study and higher precision than that of an active surveillance cohort study with comparable sample sizes. Active surveillance studies can also be used to validate estimates of VE against medically-attended influenza from TN studies.

Current estimates of influenza VE evaluate the vaccine's direct protective effects against symptomatic (or medically-attended) influenza. Future research should focus on better estimates of the indirect effects of vaccination including the impact of the vaccine against various outcomes. Better estimates of the incidence of asymptomatic cases can be generated using an active surveillance cohort in which all individuals are tested for influenza every week or two. Additionally, future research should focus on the more specific biological effects of vaccination and how the vaccine interacts with the immune system. Specifically, little is known about how influenza vaccination impacts an individual's infectiousness, how the vaccine wanes over time, and the impact of repeated vaccination. To assess the latter, multi-season cohort studies can be conducted to obtain more reliable estimates of the effect of vaccination and infection in previous seasons on the current year's VE. With a better understanding of how current vaccination policy (i.e., annual vaccination) corresponds to protection, we can confirm or adjust vaccination schedules and recommendations.

With the continued improvement of computational and biomedical technology, the next decade will see improved understanding of the biological effects of vaccination through improved modelling and more sensitive biological tests. For example, an individual's B cell population begins as a collection of naïve cells that differentiate in response to exposures throughout an individual's life. Studies have been performed to investigate the differentiation of human B cells after exposure to influenza virus (154, 155). Using this information can improve models of antibody kinetics following infection (103) and be extended to better understand the immune response to vaccination. A more complete understanding of the immunological response to influenza vaccines can then be used to inform the design of VE studies for more accurate measures of influenza VE. Additionally, progress is being made toward the creation of a universal influenza vaccine which will

provide multi-season protection (156). Once a universal vaccine is created, it will be important to continue monitoring VE from season to season to make sure there are not mutant viruses that escape the universal vaccine.

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Article Highlights

1. Influenza vaccination remains the most effective way to protect against influenza infection and evidence continues to suggest that influenza vaccines are protective against infection; however, estimates of VE vary depending on the season, vaccine type, study design, and statistical methodology employed.
2. Challenges in estimating influenza VE are numerous and complex.
3. Further studies are needed to disentangle immunological processes from artefacts of observational studies with regard to the effects of repeat vaccination on estimates of VE and waning of VE over time.
4. A knowledge gap still exists regarding the proportion of asymptomatic cases of influenza virus infection and the impact of influenza vaccines on individuals who do not develop symptoms.
5. While the test-negative design has become the most common study design used for the estimation of influenza VE, this study design only provides estimates of VE against medically-attended influenza. Medically-attended influenza cases represent a fraction of total influenza virus infections and cases. It is important to design and implement studies that determine vaccine effectiveness against other outcomes, such as symptomatic influenza.

Table 1.

Outcomes of interest against which vaccine effectiveness may be estimated.

Outcome of interest	Definition	Challenges
Asymptomatic influenza	Influenza virus infection that does not result in ARI	<ul style="list-style-type: none"> Asymptomatic individuals are hard to detect because they are not captured by existing surveillance networks Studies designed to estimate VE against asymptomatic influenza are difficult and expensive
Symptomatic influenza	Influenza virus that results in ARI	<ul style="list-style-type: none"> Individuals may not be captured by existing surveillance networks unless they seek medical care for their ARI Studies designed to estimate VE against symptomatic influenza are less common and expensive
Medically-Attended influenza	Influenza virus infection that results in ARI for which a person seeks medical care	<ul style="list-style-type: none"> Routinely captured by surveillance networks, but may not be representative of the full population VE estimates may be biased if clinicians do not test individuals for influenza using the same criteria
Hospitalizations/severe illness	Influenza virus infection that results in ARI and requires hospitalization or results in severe complication	<ul style="list-style-type: none"> At the time of hospitalization or severe complication some patients may no longer be infected (and therefore test negative for influenza infection)

ARI – acute respiratory illness; VE – vaccine effectiveness

Table 2.

Study designs used for the estimation of influenza vaccine effectiveness.

Study	Cases	Non-cases/controls	Features
Active surveillance cohort	Individuals in the cohort who report ARI and test positive for influenza virus infection	All other members of the cohort	<ul style="list-style-type: none"> • Capture any symptomatic illness • Expensive • Not affected by confounding due to propensity to seek medical care • Allows for collection of information on timing of infection and vaccination
Passive surveillance cohort	Individuals in the cohort who seek medical care for ARI and test positive for influenza virus infection	All other members of the cohort	<ul style="list-style-type: none"> • Less expensive than active surveillance cohort studies • Non-cases are representative of the entire population
Test-negative	Members of the study population who seek medical care for ARI and test positive for influenza virus infection	Members of the study population who seek medical care for an ARI and test negative for influenza virus infection	<ul style="list-style-type: none"> • Easy to incorporate into existing surveillance networks • Controls for health care seeking behavior • VE estimates may not be generalizable to entire population
Traditional case-control	Members of the study population who seek medical care for an ARI and test positive for influenza virus infection	Randomly selected individuals from the study population who did not develop an ARI throughout the study	<ul style="list-style-type: none"> • Historically used to estimate influenza VE when randomized clinical trials not feasible • Cases and controls selected from different populations