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The role of asymptomatic infections in influenza transmission: what do we really know

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Abstract

Before the COVID-19 pandemic, the role of asymptomatic influenza virus infections in influenza transmission was uncertain. However, the importance of asymptomatic infection with SARS-CoV-2 for onward transmission of COVID-19 has led experts to question whether the role of asymptomatic influenza virus infections in transmission had been underappreciated. We discuss the existing evidence on the frequency of asymptomatic influenza virus infections, the extent to which they contribute to infection transmission, and remaining knowledge gaps. We propose priority areas for further evaluation, study designs, and case definitions to address existing knowledge gaps.

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Contributors

MPM developed the methodology, conducted the investigation, wrote the original draft, and reviewed and edited the Personal View. SEM conducted the investigation, wrote the original draft, and reviewed and edited this Peronal View. MAR and WK reviewed and edited the paper. AMS conceptualised this study, developed the methodology, and reviewed and edited the paper. MB, WWD, and CR conceptualised, reviewed, and edited the Personal View. SJO was responsible for conceptualisation, supervision, reviewing, and editing the paper. The findings and conclusions in this Personal View are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention.

Declaration of interests

We declare no competing interests.

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For **Influenza Specimen Collection** see https://www.cdc.gov/flu/pdf/professionals/flu-specimen-collection-poster.pdf

Introduction

Influenza viruses cause respiratory infections that range from asymptomatic to severe and contribute to morbidity and mortality globally. Effective influenza control relies on vaccinations and individuals using control measures when they are infectious.[1] Infectiousness precedes symptom onset by 1–2 days (presymptomatic infection), and typically lasts for up to 1 week. The use of pharmaceutical and non-pharmaceutical interventions for interrupting influenza transmission largely relies on recognising symptoms. Although the presymptomatic period poses a brief window of infectiousness before illness detection, asymptomatic influenza virus infections (laboratory-confirmed infections that never progress to overt clinical signs and symptoms) also pose a threat to onward transmission. Therefore, the extent and frequency with which asymptomatic influenza virus infections contribute to onward transmission has important implications for influenza prevention and control.

Before the COVID-19 pandemic, the role of asymptomatic infections in influenza virus transmission was not firmly established.[2–4] During the COVID-19 pandemic, the public health community took several months to recognise the contribution of asymptomatic SARS-CoV-2 infections to transmission of COVID-19.[5–7] The implementation of widespread testing and strict quarantine procedures for close contacts, including rigorous symptom monitoring and frequent testing, allowed for detection of SARS-CoV-2 transmission from asymptomatic individuals in ways that had not been logistically feasible for influenza virus.[8–11] Realising the importance of asymptomatic SARS-CoV-2 infection has reignited discussions about the contribution of asymptomatic infections to influenza virus transmission and how it might affect the effectiveness of influenza control measures.

In 2009, the WHO Public Health Research Agenda for Influenza acknowledged the poor understanding of the risk of transmission associated with different clinical presentations of influenza including asymptomatic and subclinical infections.[12] However, a 2017 update of this agenda downgraded the importance of asymptomatic influenza virus infection research, citing, “there are no unmet public health needs or knowledge gaps in this recommendation that are relevant to [limiting the spread of pandemic, zoonotic, and seasonal epidemic influenza].[13]” Some experts have stated that the contribution of asymptomatic influenza virus infections to transmission is probably minimal,[2–4] whereas others asserted that the role of asymptomatic infections in propagating transmission was uncertain.[3,14–16]

We summarise the evidence for influenza transmission from individuals with asymptomatic infections and identify opportunities for additional research on this topic. We start by considering how influenza transmission studies are designed and how design decisions affect the measurement of asymptomatic infections. Then, to understand how asymptomatic infections contribute to influenza virus transmission, we consider three questions: what proportion of influenza virus infections are asymptomatic (referred to hereafter as the asymptomatic fraction); what does viral shedding teach us about the relative infectiousness of asymptomatic infections compared with symptomatic infections; and what proportion of overall influenza virus transmission is attributable to asymptomatic index infections (referred to hereafter as asymptomatic transmission)?

How asymptomatic infections are studied and how study design influences findings

Studying asymptomatic infections is inherently difficult and costly. People with asymptomatic infections do not seek testing or care and normally go undetected under traditional public-health surveillance. Therefore, subclinical infection researchers must intentionally seek out individuals with such infections. Asymptomatic infections have been reported in health-care settings,[17–21] outbreak settings,[22] and in case reports.[23] Most often, researchers have studied influenza transmission through household studies, in which index patients are identified and exposed household members are monitored closely for infection.[24]

Evidence for the presence of asymptomatic infections began to accrue soon after the influenza virus was discovered in the early 1930s.[25,26] Early studies noted inapparent infections, in which an influenza antibody response was detected but febrile illness was not recorded.[27–29] During this period, longitudinal studies among military personnel improved the understanding of influenza virus transmission.[30] After World War 2, the director of these military studies, John Dingle, applied these research methods to civilian populations and the household study design evolved.[30,31] Community household studies done over several decades were established in Cleveland, OH, USA; Tecumseh, MI, USA; Seattle, WA, USA; areas of MD and VA; and the UK.[30,32–35] The prospective study of households provided early preliminary data on asymptomatic infections. However, because studying the asymptomatic fraction and asymptomatic transmission was never the primary objective of these studies, their results left some gaps in our understanding of asymptomatic infections.

Whether designing household studies or otherwise, decisions made during study design affect the detection of asymptomatic infections.[36] Three key design aspects include: the definitions of asymptomatic and symptomatic infection, the quality of symptom assessment, and the choice of laboratory assay (table).

Case definitions

Consistent case definitions are needed to produce comparable results. As researchers of the Cleveland household studies observed in 1953, “the differentiation between a state of ‘health’ and one of ‘illness’ is difficult, particularly when consideration is given to minor illnesses.”[32] Most influenza surveillance systems use symptomatic case definitions, (eg, acute respiratory infection, influenza-like illness, or severe acute respiratory infection). Many research studies do not explicitly define asymptomatic infections. When defined, asymptomatic infections have often been classified as those with no symptoms (table). However, occasionally studies define asymptomatic infections as not meeting a symptomatic case definition, which groups asymptomatic and paucisymptomatic infections (infections with symptoms that do not meet traditional surveillance case definitions like influenza-like illness) together.[43,47] A wide lexicon has evolved to describe symptomatic infections that fall outside of surveillance case definitions, including paucisymptomatic,[14,17,49] oligosymptomatic,[21] inapparent,[27,41] subclinical,[4,23,43] and non-ill.[30] Accurately capturing asymptomatic and paucisymptomatic infections is important to estimate the true number

of influenza virus infections, because many laboratory-confirmed influenza infections do not meet surveillance case definitions, and many individuals who meet these case definitions might not seek medical care.[49,50] Furthermore, because asymptomatic and paucisymptomatic infections occur with different frequency and contribute to influenza transmission to a different extent, it is important for research studies to measure these types of infections separately.

Quality of symptom assessment

After establishing a consistent asymptomatic case definition, consideration should be given to quality of symptom assessment, which affects the number of asymptomatic infections identified in a study. Quality of symptom assessment refers to the timing and frequency of symptom assessment and the number of symptoms assessed. Regarding timing, cross-sectional, and retrospective study designs both pose challenges for detecting asymptomatic infections. Cross-sectional studies that assess symptoms concurrent with influenza testing, will misclassify some presymptomatic infections as asymptomatic.[20] Retrospective studies might be subject to poor symptom recall. In a retrospective serological study in Scotland, researchers tested influenza antibody titres before and after an influenza epidemic. At the end of the epidemic, participants were asked to recall any respiratory symptoms during the preceding 4 months.[18] Using this design, symptoms are probably under-reported compared with studies assessing symptoms prospectively with a daily log. From a risk analysis approach, under-reporting might be less concerning if poor recall is equally distributed between infected and non-infected people (ie, non-differential), but if the aim is to estimate the frequency of asymptomatic infection symptoms under-reporting can be problematic. The frequency of symptom assessment (eg, daily or weekly) along with the training and supervision of field workers, use of required data entry fields, and reminders to participants to report mild symptoms (eg, rhinorrhoea), can affect the number of asymptomatic infections identified. A study from South Africa involved twice weekly, trained field staff household visits to directly observe participants for signs of infection and inspect patients' symptom diaries for completion to reduce recall bias and under-reporting.[37] Finally, the number of asymptomatic infections identified is subject to the number of symptoms assessed, which ranged from four to 11 symptoms in the studies summarised in the table. In a household study by Cowling and colleagues in Hong Kong, researchers used three definitions of clinical influenza, varying the number of symptoms in each definition.[38] Secondary attack ratios increased from 5% to 18% when the number of symptoms in the definition increased from three to seven.

Laboratory assay

The third aspect of study design that affects detection of asymptomatic infections is the choice of influenza laboratory assay. Influenza detection assays encompass mainly two categories—serological (antibody) tests and nucleic acid detection tests (eg, RT-PCR). Serological assays detect previous influenza virus infection, whereas RT-PCR detects nucleic acid fragments at a single point in time. Different serological assays can be used, including haemagglutination inhibition and micro-neutralisation. Findings from different serological studies are difficult to compare because serological assays are strain-specific for influenza virus. The use of standardised reagents and protocols are important to ensure

reproducible results.[51,52] Serological studies are used to detect seroconversion between two timepoints, such as before and after an influenza epidemic and, when combined with symptom assessment, can be used to detect asymptomatic infections.

To detect acute infections and to make inferences on the direction of transmission between individuals with asymptomatic or symptomatic infection, nucleic acid detection tests should be used. Newer detection tools under investigation might help differentiate true infection from transient environmental contamination,[53] which is particularly relevant for studies of individuals who show no symptoms.

In studies that use both serological and nucleic acid detection tests, researchers should apply laboratory testing and case definitions equally to asymptomatic and symptomatic infections. One design used in several studies involved serology tests for all participants regardless of their symptoms, but doing RT-PCR only for symptomatic participants.[17,19,54] Cases were defined as positive by either serology or RT-PCR. This approach to estimating the asymptomatic fraction is problematic because symptomatic infections have two detection methods (serology and RT-PCR), whereas asymptomatic infections have only one (serology). In a study by Chen and colleagues,[54] when the influenza definition changed from positive serology alone to positive by either serology or RT-PCR, the number of symptomatic infections increased from 276 (51%) of 541, to 337 (56%) 602. Another consideration for studies relying on nucleic acid detection alone, is that the time window for detecting asymptomatic infections is shorter than that for symptomatic infections because of the shorter duration and lower concentration of viral shedding, which can cause misclassification of symptom status.[14,41]

Current estimates of the asymptomatic fraction are heterogeneous

Two systematic reviews done in 2014–15 improved our understanding of the asymptomatic fraction.[55] Leung and colleagues[55] separated asymptomatic fraction estimates by study type —first, outbreak investigations that used mainly RT-PCR (19 estimates from 11 studies with a pooled mean of 16%, 95% CI 13–19) and then, cohort studies that used mainly serology (19 studies). Estimates from cohort studies were too heterogeneous (ranging from 0–100%) to calculate a pooled mean estimate. In their definition of asymptomatic infection, Leung and colleagues included infections without any signs or symptoms and symptomatic infections that did not meet symptomatic criteria as defined by individual studies. Furuya-Kanamori and colleagues[56] reported asymptomatic (total absence of symptoms) and subclinical (illness not meeting individual studies' symptomatic definition) results separately. The authors found 22 estimates of asymptomatic infection from 19 studies and calculated a pooled asymptomatic percentage of 19% (95% CI 5–36).

Both reviews commented on the marked heterogeneity among the reviewed studies. In addition to the study design factors, there are inherent differences in influenza type and subtype, geographical variability, seasonal changes, host age, host immunity and susceptibility, environmental factors, and exposure dose and route, which could all contribute to true variability in the proportion of asymptomatic influenza virus infections. Furuya-Kanamori and colleagues investigated the underlying reasons for the heterogeneity

in estimates of the proportion of asymptomatic infections using multivariable regression, but could not explain most of the observed variance. The choice of influenza laboratory assay, which appeared to explain some of the variability in the Leung and colleagues' review, did not explain much of the variance in the multivariable regression model.

Studies have clearly shown that asymptomatic influenza virus infections exist and might constitute a sizeable proportion of all infections. Although the exact asymptomatic fraction remains undefined, variability in study design, influenza virus characteristics, environmental conditions, and host factors make it unlikely that additional studies or reviews would produce a more reliable estimate without controlling for these factors, as asymptomatic SARS-CoV-2 meta-analyses have shown.[57]

What we have learned from viral shedding

Unlike the asymptomatic fraction or asymptomatic transmission, viral shedding is simple to measure. Viral shedding is important because it serves as a stepping stone to understand whether and how much transmission occurs from individuals with asymptomatic infections. Viral shedding is usually defined as the detection of influenza nucleic acid in a respiratory specimen and can be measured as shedding duration (number of days when any viral RNA is detected) or as viral load (as measured by quantitative RT-PCR or viral culture).[58] Quantitative RT-PCR is not a reliable measure of virus infectivity.[59] In ferret models, viral shedding and transmission have been documented during presymptomatic influenza virus infection;[60] however, drawing conclusions from ferret models is difficult because ferrets and humans differ in how symptoms are measured and in their exposure history to influenza.[61,62]

Human challenge studies, in which volunteers are intentionally exposed to influenza virus and monitored for infection, offer a unique opportunity to study viral shedding during asymptomatic infections in a controlled setting. In a review of human challenge studies, involving 522 participants from 38 subgroups, asymptomatic infections were common.[63] However, although a third of volunteers across all studies were asymptomatic, very few studies reported data on viral shedding in asymptomatic infections. In two studies involving 76 participants, volunteers with baseline haemagglutination inhibition titres greater than 1:24, which might suggest previous exposure to influenza, were more likely to have an asymptomatic infection than volunteers with lower titres. In another two studies, involving 25 participants, the mean viral load was 2–3 log10 times higher in participants with symptomatic infections than in individuals with asymptomatic infections. Extrapolating findings from challenge studies to real-world settings is challenging because the highly controlled participant inclusion criteria and the dose, route, and timing of exposure are not representative of natural infections. Findings should be confirmed in observational studies.

A review of viral shedding in influenza A(H1N1)pdm09 infections by Fielding and colleagues[64] examined clinical severity as a primary outcome. Among 22 studies reviewed, a single household study measured viral shedding in asymptomatic infections.[41] Asymptomatic infection was not defined explicitly but presumably referred to participants who reported none of ten symptoms assessed during twice-weekly site visits. In 12

asymptomatic patients, the number of days of detectable viral RNA by RT-PCR was shorter than for all participants.

A few studies not reviewed by Fielding and colleagues provide additional evidence that viral shedding is detectable during asymptomatic influenza virus infections and is probably lower than in symptomatic infections. Of three small studies (fewer than ten participants), two found median viral load or peak viral titres to be lower in asymptomatic than symptomatic patients,[4,48] whereas the third study found no difference between the groups.[47] One of these small studies found no difference in viral shedding duration between asymptomatic and symptomatic infections.[48] A larger study examined viral shedding in 25 individuals with asymptomatic infections identified from several household transmission studies done during 6 years.[14] Asymptomatic infections had a significantly shorter duration of viral shedding and lower viral RNA copies per mL as measured by quantitative RT-PCR for most influenza virus types and subtypes, although statistical quantities (eg, SD) for the comparison of viral RNA copies per mL were not reported. In the largest study of 210 asymptomatic index infections in households, multivariable analyses found that longer shedding duration (more than 3 days) was associated with having a symptomatic infection.[37]

Overall, despite small sample sizes, viral shedding is detectable in asymptomatic infections and is probably lower in concentration and shorter in duration than in symptomatic infections. Based on our knowledge of viral shedding, the possibility of influenza transmission during asymptomatic infections is plausible and might suggest that individuals with asymptomatic infections transmit infection less frequently than individuals with symptomatic infections.

Evidence of transmission from asymptomatic individuals

Because of the large investment needed to assess transmission from asymptomatic individuals, few studies have attempted to investigate this issue. One study in South Africa by Cohen and colleagues measured asymptomatic transmission directly using a prospective household study.[37] Household members were enrolled and tested for influenza twice per week, regardless of symptoms, using RT-PCR. The study enrolled 1116 individuals from 287 households and identified 478 index influenza virus infections, including 210 (44%) of 478 asymptomatic infections. The authors reported that 6% of household members developed influenza after contact with an individual with asymptomatic infection, in contrast to 14% infected after contact with a symptomatic individual. In total, about a quarter of all secondary infections were attributable to transmission from asymptomatic individuals. Whether the secondary infections acquired from asymptomatic index cases were less clinically severe than secondary infections acquired from symptomatic index cases was not reported.

The study in South Africa appears to be unique in investigating asymptomatic transmission directly. Two small studies reported on outcomes after exposure to individuals with asymptomatic influenza virus infection.[21,43] Neither study was designed to study asymptomatic influenza transmission as a primary outcome, and both monitored a small number of asymptomatic infections (12 and 4 infections, respectively). The nature of close

contact in these studies differed from household exposure. In the study by Pang and colleagues[43] the most common contact was as an airline passenger, and in the study by Tamò and colleagues,[21] contact occurred in health-care settings. Neither study found any secondary transmission from asymptomatic index cases but might have been underpowered for this question.

One approach by researchers in Hong Kong used existing data from previous household studies to model the relative infectiousness of asymptomatic and symptomatic influenza virus infections.[65] With this approach, the researchers estimated that asymptomatic infections were 0·57 (95% CI 0·11–1·54) times as transmissible as symptomatic infections, consistent with the observational study estimates from South Africa. The authors concluded that additional data would be beneficial to reduce uncertainty in this estimate and better understand the contribution of asymptomatic infections to influenza virus transmission.

Household cohort studies with the same recruitment design used by Cohen and colleagues have been done by other researchers,[39,41,48,66] including the early studies done in Cleveland and Tecumseh.[32,67] The household study design used by Cohen and colleagues, however, differed from previous household studies in that the index case was identified through routine screening of household members regardless of their symptoms. Although this modification substantially increases the number of tests done and thus the study costs, identifying symptomatic and asymptomatic index cases allows for the study of influenza virus transmission from asymptomatic individuals. Although the increased costs are important to consider, future studies using a similar design could be feasible. Modelling studies, which depend on (scarce) input data, offer a less resource-intensive option for further study.

Public health implications

The implementation of timely and effective interventions against influenza virus transmission is essential to reducing the morbidity and mortality of seasonal and pandemic influenza. Effective influenza control depends on identifying individuals who are infectious and using protective measures to prevent further transmission. Detecting influenza and preventing transmission from symptomatic individuals is considerably more straightforward than for individuals who are asymptomatic, presymptomatic, or paucisymptomatic. Influenza detection strategies include monitoring surveillance systems for increases in influenza-like illness and increasing testing for symptomatic individuals. These detection methods are accompanied by transmission prevention strategies, including non-pharmaceutical (isolation, masking, and handwashing) and pharmaceutical interventions that are predominantly applied to patients with symptomatic infections.[68]

If a sizeable proportion of influenza transmission is attributable to asymptomatic, presymptomatic, and paucisymptomatic infections, then additional detection and prevention measures that are independent of symptom development (eg, vaccination) become even more important. Other strategies to prevent transmission from individuals with no or mild symptoms, include staying home when exposed to influenza, increased testing of asymptomatic individuals, school closures, or masking recommendations for people with

no symptoms. When these and other strategies were implemented for COVID-19, influenza virus circulation declined substantially;[69] however, these measures can be disruptive and are typically reserved for pandemics posing substantial threat.[1] Although asymptomatic, presymptomatic, and paucisymptomatic infections have similar disease control implications, these three types of infections should be distinguished in research studies because they differ in frequency and the extent to which they contribute to disease transmission. Having reliable data on the proportion of transmission resulting from asymptomatic infections is useful in selecting the most appropriate influenza control measures.

Mathematical models can be a powerful tool to identify effective influenza transmission mitigation strategies. Incorporating asymptomatic infection and transmission parameters into mathematical models generally requires defining two important values: the proportion of infections that are asymptomatic and the relative infectiousness of asymptomatic compared with symptomatic individuals. Assumptions for these values vary widely among modelling studies, from at least 20–60% and 36–100%, respectively.[70–74] Typically, the chosen values are assumed invariant across age and other factors (such as vaccination status), but there are exceptions.[73,75] The variation in assumptions of asymptomatic infection and relative transmission is unsurprising, given the uncertainty and underlying heterogeneity in estimates from epidemiological studies. However, sensitivity analyses can be done to investigate whether different assumptions affect model outputs.[76,77] Such analyses are crucial for any study that models the effect of mitigation measures targeting symptomatic infection, as underestimating the occurrence or intensity of asymptomatic transmission will overestimate the effectiveness of these interventions.[78,79]

To properly assess the effect of asymptomatic influenza virus transmission and develop accurate disease models, additional supporting evidence is needed. Estimates of the asymptomatic fraction come largely from post-hoc analyses, which results in underpowered estimates and creates challenges in pooling results because of the heterogeneity in study design. Estimates of asymptomatic transmission are even fewer. In case additional studies on asymptomatic transmission are done, they should be designed intentionally so that findings can be compared and combined across studies. Some considerations for study design, drawn from COVID-19 and influenza studies, are presented in the panel.[55–57] Key considerations include incorporating asymptomatic transmission as a primary objective (rather than as a post-hoc analysis), utilising consistent case definitions and approaches to symptom assessment, and ensuring adequate sample size. Although some study design aspects require substantial resources, modelling studies offer a lower-cost option that takes advantage of existing data to inform our understanding of asymptomatic transmission.[65,78] However, care must be taken to ensure parameter identifiability when fitting multi-parameter models to data. Multiplex assays that detect multiple pathogens (eg, influenza viruses, SARS-CoV-2, and respiratory syncytial virus) do not reduce study costs, but can increase the yield of information gained and make the effort of study staff and participants worthwhile. A standardised approach across studies can help to answer remaining questions about the probability of asymptomatic transmission under different host, environment, and viral conditions.

Conclusions

The COVID-19 pandemic has prompted us to reconsider the role of asymptomatic infections in influenza transmission. Although estimates of the asymptomatic fraction are heterogeneous, there is general agreement that asymptomatic influenza virus infections occur, cause detectable viral shedding, and result in onward transmission (as shown in at least one study).[37] The question of the proportion of transmission attributable to asymptomatic infections remains largely unanswered and is possibly variable. Studies in the last few years show that study designs that allow investigation of this proportion are feasible but could require substantial effort. Future studies intentionally designed to measure asymptomatic transmission could help to inform influenza modelling and design increasingly effective influenza control strategies. Exploration of old datasets, as done by Tsang and colleagues,[65] is a less costly method. Inclusion of asymptomatic transmission in the global influenza research agenda should be revisited.

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Panel: Considerations in the design of studies measuring influenza virus transmission from asymptomatic individuals

Population

- Select participants who are representative of the population of interest in terms of demography, geographical location, socioeconomical situation, and clinical condition. Some studies (eg, by Jackson and colleagues), use probability sampling methods to improve representativeness of the sampled population.[41]

Primary objective

- Ensure that measurement of transmission from asymptomatic individuals (not just detection of asymptomatic infections) is the primary objective when designing the study. Studies done in different contexts (geographies, populations, underlying immunity, and influenza types and subtypes) would be useful to improve understanding of asymptomatic transmission.

Study design

- Plan to collect symptom data prospectively. When possible, avoid retrospective designs, which are subject to recall bias, and cross-sectional designs, which cannot distinguish asymptomatic from presymptomatic infections.
- For household studies, consider whether a case-ascertained study or a cohort study design is more appropriate. In case-ascertained studies, households are enrolled after the index patient is identified (eg, from a primary health clinic).[80] In household cohort studies, households are selected at the beginning of the study and tested regularly to identify index patients.[78] In either design, index patients must be identified regardless of symptoms if transmission from asymptomatic index patients is to be studied. Household cohort studies, including the study reported by Cohen and colleagues,[37] might be a more suitable design to identify asymptomatic index patients than a case-ascertained design.
- Consider the substantial burden of prospective data collection on participants and simplify data and specimen collection to minimise participant dropout. Seek input from community members and leaders on acceptable study procedures.
- Explore whether existing datasets include sufficient information to model the probability of asymptomatic transmission[65]

Case definitions

- Use a standard definition of asymptomatic influenza virus infection, such as a laboratory-confirmed infection with complete absence of symptoms during the entire incubation period (usually 1–4 days but in rare cases up to 7 days from exposure).[81]

- Distinguish asymptomatic cases from paucisymptomatic cases, which differ in their frequency and relative transmissibility compared with symptomatic cases (eg, cases meeting influenza-like illness case definition).
- Consider using influenza virus subtyping or defining a time interval, such as 2 weeks[37] or 35 days,[82] between positive tests to define repeat infections

Sample size

- Account for assumptions about the expected proportion of the population to be infected, the expected proportion of index cases that will be asymptomatic, the expected proportion of asymptomatic infections that will lead to transmission, the expected proportion of close contacts lost to follow-up, within-household correlation, and any design effect related to the sampling design. See examples of sample size calculations from studies published by Simmerman and colleagues[45]and Benet and colleagues.[17]

Symptom assessment

- Include a comprehensive list of possible symptoms and record symptoms prospectively and frequently to minimise recall bias. Influenza symptoms can include fever, feeling feverish, or chills; cough; sore throat; runny or stuffy nose; muscle or body aches; headaches; fatigue (tiredness); vomiting; or diarrhoea (see US Centers for Disease Control and Prevention Flu Symptoms & Diagnosis).
- Schedule data collection trainings and provide close supervision for participants and field staff.

Testing algorithm

- Ensure that testing to identify index cases and secondary cases is done equally on participants regardless of symptoms, to ensure that symptomatic and asymptomatic cases have an equal probability of detection.
- For secondary cases, test individuals with close contact with infected index cases frequently. Most studies have tested exposed individuals every few days or twice weekly (table). Klick and colleagues reviewed the optimal timing for testing exposed individuals to identified secondary infections.[80]

Specimen collection

- Identify the specimen collection method (eg, nasopharyngeal swab, nasal swab, nasal wash, throat swab, or a combination) and materials (eg, swab type and viral transport medium).
- Review recommended materials and procedures for different types of collection methods (see Influenza Specimen Collection).
- Standardise specimen collection procedures across studies and seasons if multiple studies are planned.

- For larger studies, consider first validating the optimal specimen collection method as done by Suess and colleagues.[46]

Laboratory assay

- Select assays that detect current infection (RT-PCR or viral culture), rather than assays that identify previous exposure (serology), when assessing the direction of transmission (from an index case to a secondary case) in observational studies. Serological assays alone are insufficient to determine the timing or direction of transmission between cases in observational studies. Serological data can contribute to modelling studies to understand influenza transmission dynamics.[65]
- Use assays with high specificity to minimise detection of false positive influenza infections, particularly in asymptomatic patients where the prevalence of influenza virus infection is lower than in symptomatic patients.

Analysis

- Ensure that appropriate data are collected and reported to allow for stratified or multivariable analyses, including age, race, ethnicity, influenza type and subtype, vaccination status, underlying medical conditions, and household characteristics

Table:

Variability in asymptomatic and symptomatic influenza virus infection definitions and detection in representative studies

	Population and setting	Number of symptoms assessed	Symptoms assessed	Duration of symptom monitoring	Asymptomatic definition(s)	Symptomatic definition(s)	Symptom assessment	Influenza assay	Timepoints for influenza assessment
Benet et al (2021)[17]	Health-care workers from five university hospitals	Unknown	Not reported	One influenza season	No fever and absence of any signs or symptoms	ILI; paucisymptomatic defined as one or more signs or symptoms but not meeting ILI definition	Daily self-collection	Seroconversion or RT-PCR	Before, during, and after influenza season (serology) and when symptomatic (RT-PCR)
Cohen et al (2021)[37]	Households in community	10	Fever, cough, difficulty breathing, sore throat, nasal congestion, chest pain, muscle aches, headache, vomiting, and diarrhoea	One visit a few days before first positive RT-PCR result through a few days after the last positive RT-PCR result	None of the ten symptoms	One symptom; two or more symptoms; ILI; one symptom, medically attended; two or more symptoms, medically attended; and ILI, medically attended	Twice-weekly questionnaire	RT-PCR	Twice weekly
Cowling et al (2008)[38]	Households with positive index case	Up to 8	Set 1: fever, headache, coryza, sore throat, myalgia, arthralgia, cough, fatigue; set 2: fever, cough, headache, sore throat, myalgia, arthralgia	9 days	Not defined (presumably no symptoms reported)	Fever or two of the seven symptoms; at least two of the five symptoms; and ILI	Daily symptom record sheet	RT-PCR or viral culture	36 h, 3 days, 6 days, and 9 days after randomisation
Elder et al (1996)[18]	Health-care workers from four acute-care hospitals	NA	Individual symptoms not assessed	One influenza season	No reported recall of self-diagnosed influenza or no reported recall of respiratory infection	Self-diagnosis of influenza; sick leave due to influenza; any respiratory infection; sick leave due to respiratory infection; and doctor diagnosis of influenza (self-reported)	Single timepoint, retrospective, self-report at the end of influenza season	Seroconversion	Before and after influenza season
Gordon and Reingold (2018)[36]	Households with positive index case	Unknown	Not reported	9–12 days after enrolment	No symptoms	Not defined	Daily	RT-PCR	Baseline and every 2–3 days for four more specimens
Hayward et al (2014)[39]	Households in community	8	Any of cough, cold, sore throat, or ILI; If yes, then feverish, headache,	One influenza season	Inferred from indirect measurement	Any ARI is cough, cold, sore throat, and ILI is fever and cough or sore throat	Weekly telephone or online survey, and daily	Seroconversion or RT-PCR	Beginning and end of each influenza season (serology) and

	Population and setting	Number of symptoms assessed	Symptoms assessed	Duration of symptom monitoring	Asymptomatic definition(s)	Symptomatic definition(s)	Symptom assessment	Influenza assay	Timepoints for influenza assessment
			muscle aches, cough, sore throat, runny nose, blocked nose, and sneezing				symptom diary while symptomatic		when symptomatic (RT-PCR)
Ip et al (2017)[14]	Households with positive index case	7	Fever, headache, myalgia, cough, sore throat, runny nose, and sputum	6 days after enrolment	None of the seven symptoms	ARI is two or more symptoms; ILI is fever plus cough or sore throat; and paucisymptomatic is one of seven signs or symptoms	Daily self-reported symptom diary	RT-PCR or viral culture	On days 0, 3, and 6
Jackson et al (2011)[40]	Households with positive index case	11	Fever, cough, sore throat, headache, difficulty breathing, fatigue, muscle pain, nausea, vomiting, diarrhoea, and runny nose	Up to 30 days	None of the 11 symptoms	ARI is two or more of fever, cough, sore throat, or runny nose; and ILI is fever with cough or sore throat	Questionnaire administered at two household visits, 1–2 months apart	Seroconversion	Twice, 1–2 months apart
Lau et al (2010)[4]	Households with positive index case	7	Fever, headache, myalgia, sore throat, runny nose, cough, and phlegm	Approximately 7 days after enrolment	None of the seven symptoms	ARI is two or more of the seven symptoms; subclinical is only one symptom	Daily symptom diary	RT-PCR or viral culture	On days 0, 3, and 6
Loeb et al (2009)[19]	Health-care workers from eight tertiary care hospitals	11	Fever, cough, nasal congestion, sore throat, headache, sinus problems, muscle aches, fatigue, earache, ear infection, and chills	Duration of an influenza season	None of the 11 symptoms	ILI is cough and fever	Twice-weekly web-based questionnaire	Seroconversion or RT-PCR	Beginning and end of influenza season (serology) and when symptomatic (RT-PCR)
Loeb et al (2012)[41]	All households from multiple community colonies	10	Fever, cough, nasal congestion, sore throat, headache, sinus problems, muscle aches, fatigue, earache, and chills	Up to 4 weeks	None of the ten symptoms	ARI is two or more of the ten symptoms	Twice per week	RT-PCR	Daily for one week, then every other day for up to 3 weeks
Maier et al (2018)[42]	Households with positive index case	4	Fever, sore throat, cough, and runny nose	10–13 days once a symptomatic patient was identified in the household	None of the four symptoms	ARI is two or more of the four symptoms; paucisymptomatic is one symptom, not including fever; and ILI is fever plus cough or sore throat	Daily symptom diary and temperature monitoring	RT-PCR	Up to five times during 10–13-day follow-up

	Population and setting	Number of symptoms assessed	Symptoms assessed	Duration of symptom monitoring	Asymptomatic definition(s)	Symptomatic definition(s)	Symptom assessment	Influenza assay	Timepoints for influenza assessment
Melchior et al (2015)[20]	Multiple	Unknown	Not reported	Cross-sectional	Absence of respiratory symptoms in the 15 days before sample collection	Acute respiratory infection (not defined) within a week before sample collection	Cross-sectional assessment	RT-PCR	Cross-sectional
Pang et al (2011)[43]	People in quarantine identified by multiple screening methods	5	Fever, cough, sore throat, nasal congestion, and rhinorrhoea	7 days	Not defined; subclinical and asymptomatic terms are used interchangeably	Not defined	Not defined	RT-PCR	At baseline regardless of symptoms; a second specimen collected if symptomatic
Papenburg et al (2010)[44]	Households with a positive index case	4	Fever, cough, sore throat, and rhinorrhoea	Up to 4 weeks	Completely asymptomatic	ARI is two or more of the four symptoms; ILI is fever and cough or sore throat	Questionnaire on days 0, 8, and 11, and after 3–4 weeks	Seroconversion or RT-PCR	Baseline (RT-PCR and serology) and at 3–4 weeks (serology)
Sagrera et al (2002)[22]	Outbreak in neonatal intensive care unit	Open-ended	Not defined but both respiratory and gastrointestinal symptoms are mentioned	48 h before and after obtaining a specimen positive for influenza A	Not defined (presumably no symptoms or symptoms attributable to other causes)	Any type of symptomatology not explained by other causes	Patients monitored in neonatal intensive care unit	Immunofluorescence antibody screen or viral culture	Every 3 days or when symptomatic
Simmerman et al (2011)[45]	Households with positive, paediatric index case	Up to 8	Fever, nasal discharge or congestion, cough, conjunctivitis, respiratory distress (tachypnoea, retractions), sore throat, and new seizure	21 days	Not defined	ILI in individuals aged 2 years and older is defined as fever plus cough or sore throat; ILI in individuals younger than 2 years is fever plus at least one of seven symptoms	Daily symptom record	Seroconversion or RT-PCR	Serology on days 0 and 21 and RT-PCR on days 0, 3, and 7
Suess et al (2010)[46]	Households with positive index case	6	Fever, chills, cough, sore throat, headache, and myalgia	8 days after symptom onset of the index case	None of the six symptoms	ILI defined as fever and cough or sore throat	Daily assessment	RT-PCR	Daily up to 8 days
Suess et al (2012)[47]	Households with positive index case	4	Fever, chills, cough, and sore throat	8 days	None of the four symptoms	Symptomatic defined as having one or more of the four symptoms; ILI is fever plus cough or sore throat	Daily questionnaire	RT-PCR	4 to 8 times during an 8-day observation period
Tamò et al (2022)[21]	Health-care workers and hospitalised patients in a tertiary care hospital	8	Cough, sore throat, fever, nasal congestion, weakness, headache, loss of appetite, and myalgia	Duration of influenza season (health-care workers); duration of hospital stay plus 2 days after	Fully asymptomatic; swab positive before symptoms; and asymptomatic when test positive	Any symptom	Daily illness diary	RT-PCR	Daily for the duration of enrolment

	Population and setting	Number of symptoms assessed	Symptoms assessed	Duration of symptom monitoring	Asymptomatic definition(s)	Symptomatic definition(s)	Symptom assessment	Influenza assay	Timepoints for influenza assessment
				discharge (patients)					
Thai et al (2014)[48]	Households in a selected community	11	Sore throat, nasal congestion, runny nose, sneezing, dry cough, wet cough, headache, diarrhoea, myalgia, fever, and wheeze	15 days	None of the 11 symptoms	Any symptom	Daily assessment	RT-PCR	Daily for 10–15 days after exposure (RT-PCR)

ARI=acute respiratory illness. ILI=influenza-like illness. NA= not applicable.