Asymptomatic and presymptomatic transmission of SARS-CoV-2: A systematic review
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June 12th, 2020

Abstract:
Many of the statutes comprising the shelter-in-place and phased-reopening orders are centered around minimizing asymptomatic and presymptomatic transmission. Assumptions about the presence and relative importance of asymptomatic and presymptomatic transmission are based on case reports, the failing of quarantine measures aimed at sequestering ill patients, viral dynamic studies suggesting SARS-CoV-2 production peaks before symptoms appear, and modeling evidence that calculates serial interval between successive generations of infection. In aggregate, these data offer compelling evidence of presymptomatic and asymptomatic transmission, but individually these studies have notable shortcomings that undermine their conclusions. Conducting high quality studies with the aim of understanding the relative role of presymptomatic and asymptomatic transmission is instrumental to developing the most informed policies on reopening our cities, states, and countries. To that end, the purpose of this systematic review is to discuss the literature of asymptomatic and presymptomatic transmission, highlight limitations of recent studies, and propose experiments that, if conducted, would provide a more definitive analysis of the relative role of presymptomatic and asymptomatic transmission in the ongoing SARS-CoV-2 pandemic.

Introduction:
Understanding how SARS-CoV-2 is transmitted is a question that has been at the forefront of efforts to curtail the pandemic. On January 14, 2020, the World Health Organization announced that “there is no clear evidence of human-to-human transmission” of SARS-CoV-2. Six days later, when WHO announced evidence of human to human transmission, countries were left scrambling to enact policy to identify and isolate the ill. Only after these efforts failed, were the more comprehensive quarantine and isolation policies enacted in cities like Wuhan, China. In the absence of definitive evidence of asymptomatic transmission, these policies were made out of an abundance of caution. Understanding the temporal dynamics of SARS-CoV-2 transmissibility is key to safely and successfully reopening our cities, states, and countries until the development of an effective vaccine. Unfortunately, with over 7.25 million confirmed cases and over 413,000 deaths, there is still confusion and a dearth of adequate research around the dynamics of transmissibility of SARS-CoV-2 in the general population. On June 8th, 2020, WHO official Maria Van Kerkhove said that asymptomatic transmission of the coronavirus was "very rare." However, she later clarified this statement saying, “the available evidence from contact tracing reported by Member States [of WHO] suggests that asymptotically-infected individuals are much less likely to transmit the virus than those who develop symptoms.” Given the absence of definitive information, and because of the importance of this question, there is an urgent need to direct high quality studies towards examining asymptomatic and presymptomatic transmission of SARS-CoV-2.

Asymptomatic individuals are defined as individuals who test PCR positive, but lack symptoms that would indicate SARS-CoV-2 infection. While some individuals may go the entire course of infection and never experience symptoms, other individuals who initially present as asymptomatic may go on to develop symptoms days or weeks later. The individuals who will later develop symptoms are defined as being presymptomatic. The first large scale reporting of asymptomatic SARS-CoV-2 infection occurred on the Diamond Princess cruise ship, where an estimated 17.9% of cases on board were asymptomatic. The phenomenon of asymptomatic SARS-CoV-2 infection has since been established in multiple studies, including a UCSF study that found that 53% of individuals who tested positive were not experiencing symptoms at the time of the test. While the existence of asymptomatic cases is well understood, the
link between asymptomatic/presymptomatic cases and transmissibility is more tenuous. PCR testing can
tell us whether there is detectable virus present, but it cannot tell us whether an individual is
contagious.\textsuperscript{[7]} Infectivity in cell culture is the standard for determining whether a patient is infectious. In
the absence of viral culture data, viral load or cycle threshold (Ct) values derived from RT-PCR data is our
best proxy for the likelihood of transmission. The Ct is the number of cycles required for a signal of PCR
product to cross a determined threshold. This value is inversely proportional to the amount of target
nucleic acid or viral load in the sample – in particular, high Ct values indicate low viral load. In a study of
90 patients with SARS-CoV-2 infection, Bullard and colleagues found that virus was only successfully
isolated when Ct value was below 24.\textsuperscript{[8]}

Studies have attempted to look at viral dynamics in asymptomatic and presymptomatic individuals. One
study, from a skilled nursing facility in Kings County, Washington, found viral growth in a patient sample
with a cycle threshold (Ct) value of 34, as well as viral growth in asymptomatic and presymptomatic
individuals.\textsuperscript{[9]} However, it is problematic to extrapolate findings from patients in an elder care facility to
the general population; it is difficult to recognize early signs and symptoms of respiratory viral infections
in elderly populations, like those that would reside in a skilled nursing facility, due to impaired immune
responses associated with aging and the high prevalence of preexisting and underlying conditions, such
as chronic cough and cognitive impairments. Furthermore, elderly and infirm patients have blunted
physiological responses that may allow them to remain asymptomatic during infection. Influenza, another respiratory virus, often manifests with few or atypical symptoms in this population, resulting in
confounding of when symptoms are first reported and undermining efforts to isolate ill patients.\textsuperscript{[9]}
A second report, looking at individuals exposed during a flight from China to Frankfurt, identified one case
of asymptomatic infection and one case of presymptomatic infection with positive culture infectivity.\textsuperscript{[10]}
However, the study does not provide information about the passengers’ health or age, and there is likely
a bias to downplay mild or moderate symptoms in the context of being detained while traveling. Although these studies have attempted to look at viral dynamics in asymptomatic and
presymptomatic individuals in specific populations, to date there have been no studies that have
successfully cultured live virus from asymptomatic or presymptomatic individuals in the general
population.

Despite the absence of live virus isolation and culturing in the general population, many studies and
reports have concluded asymptomatic and presymptomatic transmission are prevalent in this
pandemic.\textsuperscript{[11]} Modeling studies that are being utilized to predict future case spread and determine the
most effective interventions are fundamentally rooted in an understanding of asymptomatic and
presymptomatic transmission.

The basis for asymptomatic and presymptomatic transmission in other viral infections.
Viral illnesses have varying transmission profiles. Seasonal influenza is characterized by having peak viral
load one day after symptom onset.\textsuperscript{[12]} Individuals have detectable levels of RNA from two days before
clinical symptoms appear to eight days afterward. Although asymptomatic and presymptomatic
individuals may shed influenza virus, studies have not determined if such people effectively transmit
influenza.\textsuperscript{[13]}

Other viral illnesses like MERS, SARS and Ebola are notable because infectivity appears to increase later
in course of illness. In the case of SARS-CoV, infectiousness peaked 7-10 days after symptom onset.\textsuperscript{[14]}
MERS-CoV concentrations peaked during the second week of illness.\textsuperscript{[15]} Ebola virus does not appear to
have presymptomatic transmission, though individuals can remain infectious for long periods of time
after symptoms resolve.\textsuperscript{[16]}
Understanding the viral dynamics and transmission profile of a virus is important because it informs the most effective outbreak curtailment strategies. In the case of SARS and Ebola, efforts aimed at sequestering the ill and contact tracing are highly effective. In the case of influenza, contact tracing must extend to the presymptomatic phase, and more aggressive prophylactic containment strategies are necessary. Efforts to curtail this SARS-CoV-2 pandemic will rely on successful contact tracing to halt further transmission. Decisions on how far back to trace contacts and if/when to test asymptomatic contacts will rely on a comprehensive understanding of asymptomatic and presymptomatic transmission.

Methods
Articles for this review were extracted from a PubMed search conducted on June 10, 2020. Articles had to either contain the phrase SARS-CoV-2 or COVID-19 as well as one of the following phrases: presymptomatic transmission, asymptomatic transmission, viral dynamics, viral kinetics, virological analysis, or serial interval. There were initially 72 results. 2 additional records were added because studies in the review referenced or analyzed their data. Reviews, correspondence, duplicate references, or articles that didn’t include measured data were excluded. Of the 35 remaining articles, the articles fell into the broad categories of (1) case reports, (2) viral dynamic studies, or (3) analysis of serial interval between linked generations of cases. Each of these broad categories will be discussed separately in a subsequent section.

![Figure 1. From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097](https://doi.org/10.1371/journal.pmed1000097)
Results of Literature Review on Evidence of Asymptomatic and Presymptomatic Transmission in SARS-CoV-2

Case Studies Suggesting Asymptomatic or Presymptomatic Transmission

| Articles | Type | Description |
|----------|------|-------------|
| Ye et al. [17] | Case Report | Family cluster of five patients. One of the patients was believed to be the source of infection, and infected others during a family reunion. Asymptomatic transmission offered as possible explanation. |
| Qiu et al. [18] | Case Report | In 304 cases from Hunan province hospitals, 5 were identified as asymptomatic. Contact tracing suggests two of these cases infected family members. |
| Huang et al. [19] | Case Report & Parameter Estimation | One 22 year old from Wuhan appears to have infected his cousin and six classmates while presymptomatic. |
| Tong et al. [20] | Case Report | Reported on two people who were infected with SARS-CoV-2 and their infections appear to have stemmed from contact with a potentially asymptomatic/presymptomatic colleague. These individuals went on to infect other members of their household. However other sources of SARS-CoV-2 infection were not ruled out. |
| Hu et al. [21] | Case Report | Contact tracing identified 24 asymptomatic COVID-19 infections in Nanjing, Jiangsu Province. One of these cases appears to be a possible source of infection in a relative, who went on to develop severe pneumonia. |
| Li et al. [22] | Case Report | Father appears to have infected his daughters, son-in-law, his son-in-law’s wardmate, and his wardmate’s family while asymptomatic. 2 family clusters of 6 patients stemming from one possible asymptomatic transmitter. |
| Wei et al. [23] | Case Report | Investigation into 157 SARS-CoV-2 infections in Singapore revealed ten cases in 7 family clusters whose presymptomatic transmission appears to have occurred. |
| Lytras et al. [24] | Case Report | Noted the relatively high rates of asymptomatic SARS-CoV-2 infection in repatriation flights in flights from Spain, Turkey, and UK. Postulates about the possibility of presymptomatic transmission. |
| Wong et al. [25] | Case Report | Identifies two asymptomatic carriers from a cluster in the Seri Petaling Mosque in Kuala Lumpur, Malaysia, who appear to have transmitted infection to others. |
| Chen et al. [26] | Case Report | Reported on a family cluster in Hubei province where it appears a parent transmitted SARS-CoV-2 infection to their children while asymptomatic. |
| Ochiai et al. [27] | Case Report | 52 obstetrical patients were tested before hospital appointments at Keio University Hospital in Tokyo, Japan. 4% were found to be asymptomatic. |

Table 1. A summary of case reports from the literature search that yielded insight into the question of asymptomatic and presymptomatic transmission.

The early literature of SARS-CoV-2 asymptomatic transmission was dominated by case reports of apparent asymptomatic transmission. A majority of these cases were individuals exposed during travel to Wuhan or other cities in Hubei Province, who later transmitted the infection to members of their household or other close contacts. [17, 18, 19, 20, 22, 26] Huang and colleagues noted a cluster of seemingly asymptomatic transmission among children, who had rapid onset of illness and various nonspecific or atypical manifestations of illness. [19] While many of these case reports took steps to ensure that those infected by asymptomatic/presymptomatic individuals did not have other plausible sources of infection, they were unable to definitively rule out other sources or community transmission. Other case reports
center around regions that were believed to not have community transmission, where exposure to other sources of infection are less likely. One example is the case of a Chinese businesswoman who appeared to have asymptotically infected some of her colleagues during a work trip in Germany. However, after publication, the supplementary material was modified because the original patient recalled that she was experiencing symptoms during her meetings with colleagues. This update to the NEJM article highlights the subjective nature of case reports documenting asymptomatic and presymptomatic transmission. It is easy for patients or practitioners to make errors when recalling or reporting symptom onset date. Another case report focuses on seven clusters in Singapore where presymptomatic transmission appeared to be the most likely explanation. This study identified 10 cases where presymptomatic transmission appeared to occur 1-3 days before symptom onset in the initial patient. While compelling, none of these cases can definitively rule out mild symptoms being present during transmission, or other sources of infection.

All case reports of presymptomatic and asymptomatic transmission are confounded by the highly subjective nature of reporting symptom onset and exposure date. Factors like age, cultural norms, and public communication about the pandemic may influence when people report their symptoms beginning. For example, an older person with chronic illness may attribute muscle and joint pain to age, whereas a younger person may call that a symptom. Additionally, as the pandemic has progressed, our categorization of what is considered a symptom has expanded. In February, the WHO said symptoms of COVID-19 included fever, dry cough, fatigue, sputum production, shortness of breath, sore throat, headache, myalgia or arthralgia, chills, nausea or vomiting, nasal congestion, diarrhea, hemoptysis, and conjunctival congestion. In late February, Mao and colleagues first reported that anosmia, or loss of sense of smell, were symptoms of COVID-19, and this finding was supported in additional research. On April 17th, the WHO added loss of smell or taste as well as rash and skin discolorations of fingers and toes as additional symptoms of COVID-19. Knowledge of these changing definitions, differing levels of chronic illness, and varying levels of symptom awareness will alter when individuals first report experiencing symptoms.

An additional report included in this keyword search inferred the possibility of asymptomatic transmission from positive RT-PCR tests in presymptomatic and asymptomatic individuals. Lytras and colleagues noted a high prevalence of SARS-CoV-2 infection in asymptomatic cases in repatriation flights to Greece. While this study does support the well-documented phenomenon of asymptomatic cases, the possibility of asymptomatic transmission is a hypothetical, as a positive PCR test does not imply an individual is contagious. This study lacked insight into the feasibility of actual transmission during presymptomatic or asymptomatic infection because the authors failed to report Ct values of PCR positive individuals, did not culture virus, and did not identify possible transmission chains.

### Viral Dynamics

| Paper | Included/Excluded in Review (excluded articles are listed in appendix) | Category | Description |
|-------|-------------------------------------------------|---------|-------------|
| Wölfe et al. | Included | Viral Dynamics | Viral dynamics determined from 9 individuals from a single cluster in a single hospital in Munich, Germany. All patients were admitted after symptom onset. For most of the patients, observation appears to have begun after their viral load peaks. At the time of this review, no information was found about patient treatments. |
| Liu et al. | Included | Viral Dynamics | 76 patients admitted to the First Affiliated Hospital of Nanchang University (Nanchang, China) from Jan 21 to Feb 4, 2020. 46 cases were mild and 30 were severe. At the time of this review, no information was found about frequency or duration of sample |
Studying temporal viral dynamics allows us to predict peak infectiousness. In this review there were ten studies that measured viral temporal dynamics and kinetics of SARS-CoV-2. Eight of these studies measured viral dynamics by quantifying successive nasopharyngeal swabs in hospitalized patients. The two remaining papers focused exclusively on asymptomatic and presymptomatic individuals. From the eight studies of viral dynamics in hospitalized patients, all patients except one in the Zou et al. paper...
were symptomatic. The one asymptomatic individual in Zou et al. remained asymptomatic throughout the course of the study.

The eight studies reported viral loads were at their highest levels when observation began. Therefore, the authors of these studies concluded viral load peaks when symptoms peak. However, this discovery must be prefaced by the limitation that all patients in the studies were enrolled after symptom onset, and therefore presymptomatic viral loads were not measured. Additionally, the studies do not disclose how soon the first swab was taken after symptoms were reported; a margin of error of a day could dramatically change the viral load in patients. While the finding that viral load appears to peak soon after symptoms are detected in patients suggests that presymptomatic transmission is plausible, there is not enough information about the distribution of SARS-CoV-2 viral kinetics in presymptomatic stage to infer when infectiousness begins. Basic assumptions about the distribution will have dramatic effects on our prediction of when infectivity begins, and the specific time between symptom onset and viral load tests can dramatically change our understanding of transmissibility and infectiousness. Knowledge of the shape of the distribution will impact our responses to curtail the pandemic.

![Diagram of viral load distribution](image)

**Figure 2.** Hypothetical distributions of SARS-CoV-2 viral load. Different assumptions about the shape of the distributions will impact when and if presymptomatic transmission will occur. A line indicating the threshold of transmissibility is shown in purple, which is currently believed to be $10^6$ copies per mL. The intersection of the purple line with the various curves would show when an individual becomes contagious. In these hypothetical distributions, a normal and Weibull distribution suggest significant presymptomatic transmission, while a gamma and lognormal distribution seem to suggest limited presymptomatic transmission. However, these conclusions can change with different transmission thresholds and distribution parameters. A well-designed study will help determine the shape of this curve. While many studies concluded viral load peaks when observation begins, for almost all of the studies, a significant portion of time elapsed between when symptoms first appeared and observation began.
Wolfel and colleagues attempted to relate RT-PCR quantification of viral load with infectivity. The authors combined RT-PCR measurement with viral culturing and found that the success of virus isolation in culture depended on viral load: only samples that contained greater than $10^6$ copies per mL yielded an isolate (although Ct value was not reported in this study, He et al. reports this corresponds to a Ct value of 24). No isolates were obtained after day 8, despite continuing high viral loads. Persistent RNA detection appears to represent non-viable virus that is not infectious. This finding demonstrates that while viral load can be predictive of transmissibility, it is not a perfect correlation. The viral studies Wolfel et al., Liu et al., To et al., Young et al. and Yoon et al. were limited by small sample size. However, He et al., Liu et al., and Ding et al. have similar findings with larger sample sizes.

Despite the attempt to comprehensively profile SARS-CoV-2 kinetics, all eight of these studies were limited in their scope because they were not able to swab patients before symptom onset. An additional limitation of these studies is that many failed to specify the exact schedule with which patient swabs were collected. Only one study, To et al. mentioned a precise collection schedule that applied to all patients. It is also worth noting that nasopharyngeal swabs are an imperfect proxy for viral production. Studies on influenza have shown variability in viral load when sampling left and right nostrils and this finding will likely be similar for SARS-CoV-2. Perhaps the most important limitation of these studies is that the studies either did not specify or did not exclude individuals who were undergoing treatment. Undergoing antiviral, interferon, or steroid therapy may disrupt the natural progression of viral load. While the study by Ding and colleagues had the purpose of examining the viral kinetics during antiviral treatment, data focusing on viral load after therapeutic interventions cannot provide insight into the viral dynamics of the typical course of infection. Antiviral and interferon treatments should diminish viral replication and artificially cause viral load to peak at the start of treatment, while steroid treatment may dampen the immune response and potentially cause viral replication to increase. If the viral load data is a basis for clinical decision making, this will even further confound results because an increasing viral load would be the basis for more extensive interventions and therapeutic treatment.

There is an urgent need to study the viral kinetics in presymptomatic individuals. Kim et al. analyzed the Ct values of three presymptomatic patients and found the highest levels of virus were one to two days before symptom onset. However, this dataset is extremely small ($n=3$), and one of the patients was on the threshold of detection. Therefore, it is hard to reliably extract general trends from this limited sample. Zhou et al. studied the viral dynamics of 31 patients who were asymptomatic upon hospital admission for laboratory confirmed SARS-CoV-2 infection. Twenty-two of the patients went on to develop symptoms while nine remained asymptomatic. When comparing the viral dynamics of asymptomatic and presymptomatic individuals, Zhou et al. found asymptomatic individuals had lower Ct values, and had peak viral loads in the second week of hospitalization. Unfortunately, this data cannot be extrapolated to inform our understanding of presymptomatic viral dynamics because symptom onset date was not disclosed, and the viral load data is not shared in relation to symptom onset.

While there currently appears to be consensus that viral load appears to peak with the beginning of observation, it is important to note that these studies are still preliminary and that there is a dearth of data studying infectiousness during the presymptomatic interval. Knowledge of the shape of the distribution would be valuable to our understanding of transmissibility of SARS-CoV-2.

**Serial Interval Between Generations of Cases**

Another approach to uncovering the prevalence of presymptomatic transmission has relied on calculations of serial interval. Serial interval is defined as the time between symptom onset in the first-
generation case and the second-generation case. This method requires identification of serial cases where one individual (first-generation case) infected another individual (second-generation case). If the observed mean serial interval is shorter than the incubation period, this would support the conclusion that a significant portion of transmission may have occurred presymptomatically.

Fourteen papers in this review calculated serial interval by looking at paired cases with probable point transmission linkage. Twelve of the fourteen reports calculated serial interval by compiling data from publicly available sources such as government reports and news outlets. However, it is difficult to control for quality and bias from these publicly available reports. Additionally, these datasets are compiled from human-to-human transmission reports from different countries, jurisdictions, and points in time. It is difficult to know if standards of reporting cases or symptom onset vary by jurisdiction or time. In addition to bias or error in the publicly sourced data, all of the serial interval studies are additionally confounded by their reliance on self-reported symptom start date. As stated earlier in this paper, what is considered a symptom varies by region, culture, age, and time, and the definition of symptoms has become more expansive as time has progressed. For example, patients who notice loss of smell may have an earlier symptom start date than a patient who only reports fever and dry cough. The date reported as the onset of symptoms is also subject to error due to inherent inaccuracy of memory. Furthermore, in the datasets, the authors report the date of symptom onset rounded to the nearest day. This is especially problematic because the difference in serial interval and incubation period calculated in these studies often differed by less than a day. It is therefore not possible to ascertain if the difference between calculated serial interval and incubation period are true differences, or an artefact of rounding error.

| Paper | Included/Excluded in Review (excluded articles are listed in appendix) | Category | Description |
|-------|-------------------------------------------------|----------|-------------|
| Nishiu et al. [90] | Included | Serial Interval | Identified 28 paired cases, 18 of which were considered high quality. The data was fit to many different distributions, data in Figure 3. Is from Weibull distribution of serial intervals. Data sourced from articles and government documents. |
| Du et al. [91] | Included | Serial Interval | Identified 468 paired cases from provinces outside of Hubei Province in China. Assumed normal distribution of serial intervals. (The authors ruled out gamma or Weibull distribution). In 49 of the 468 cases, infectee appears to have symptom before infector. |
| Bi et al. [92] | Included | Serial Interval | Serial interval calculated from 48 pairs with a clear relationship between index case and secondary case. A gamma distribution of serial interval times was used. |
| Wu et al. [93] | Included | Serial Interval | Studied 48 secondary cases stemming from household transmission. Fit to lognormal distribution. Zhuhai, China. Enrolled index cases and studied their household members. |
| Ganyan et al. [94] | Included | Serial Interval | Studied 54 cases in Singapore and 135 paired cases in Tianjin, China that were part of outbreak clusters. Authors used gamma distribution. Included cases in clusters with likely but not definitive transmission links. |
| Wang K. et al. [95] | Included | Serial Interval | Serial intervals were estimated from 26 (probable: 9; certain: 17) paired data from Hong Kong Centre for Health Protection (CHP) before February 33, 2020. Data in Fig. 3 from lognormal distribution of serial intervals, but gamma distribution also examined. |
| Kwok et al. [96] | Included | Serial Interval | Enrolled 35 index cases and found 9 cases with transmission links. From these 9 cases, calculated serial interval and assumed gamma distribution of serial intervals. Patients were seen at Wuhan Union Hospital between January 5 to February 12, 2020. |
| Wang X. et al. [97] | Included | Serial Interval | Serial intervals calculated using Poisson likelihood-based (ML) method, which used a discretized gamma prior distribution. |
| You et al. [98] | Included | Serial Interval | Data sourced from 71 linked transmission cases outside Hubei Province as of March 31, 2020. |
| Author(s)          | Included Information | Data Source                        | Description                                                                 |
|--------------------|-----------------------|------------------------------------|-----------------------------------------------------------------------------|
| Böhmer et al.      | Included              | Serial Interval & Case Report      | Data sourced from one outbreak cluster and their contacts in Germany. Altogether 16 paired transmission events were reported. At the time of this review, no information was found about the distribution used. |
| He et al.          | Included              | Serial Interval and Viral Dynamics | 77 transmission pairs were sourced from publicly available information from multiple countries. Data was fitted to a gamma distribution of serial intervals. |
| Zhang et al.       | Included              | Serial Interval                    | Serial Interval calculated from 35 secondary cases stemming from 28 primary cases. Serial interval was fit to a gamma distribution of serial intervals. Data taken from provinces outside Hubei. |
| Aghaali et al.     | Included              | Serial Interval                    | Study calculated serial interval for 37 linked cases in Qom, Iran, who were identified through contact tracing. Due to limited availability of PCR tests, second generation cases were confirmed with chest CT. Authors assumed a gamma distribution of serial intervals. |
| Du et al., MedRxiv | Included              | Serial Interval                    | 339 confirmed cases of COVID-19 identified from 264 cities in mainland China prior to February 19, 2020. Data used in Fig 3. assumes normal distribution. There were 240 unique infected, and the average number of transmission events per infector is 1.41. Found household transmission led to shorter serial interval than non-household transmission inside the household (4.57 days [95% CI 3.76–5.38]) versus outside the household (5.85 days [95% CI 5.06–6.64]). |
| Son et al.         | Initially included but then excluded | Serial Interval                    | Study of patients in Buan. Authors report mean serial interval as 5.54 days [95% CI 4.08-7.01 days]. Excluded because full article was not available in English. |
| Pung et al.        | Initially included then excluded | Serial Interval                    | Study was of the first three clusters in Singapore, which identified 3 paired transmission cases. Study was excluded because no statistics on data were provided, and primary data could not be located. |
| Li et al.          | Initially included then excluded | Serial Interval                    | This study used prior assumptions from SARS-CoV data in their calculation of serial interval, therefore study was excluded. |
| Huang et al.       | Excluded from Serial Interval Data | Serial Interval and Case Report    | Data about serial interval excluded because general population was not studied. Study focused exclusively on young individuals. |

Table 3. Results of literature search that yielded insight into the question of presymptomatic transmission that pertained to serial interval. Studies that were excluded after full text analysis were also included.
Figure 3. Green line shows the reported incubation period of 5.1 days. Green shaded area shows 95% CI of incubation period as reported by Lauer et al. Data points are the mean and 95% CI on serial interval from the ten papers highlighted in this review. Confidence intervals were self-reported from each study.

Many studies of serial interval are biased towards household transmission because it is more straightforward to isolate transmission chains and rule out other sources of infection in a household setting. However, in household transmission cases, newly infected individuals will likely be exposed to a much higher dose of viral particulates than would occur in a more casual transmission case. Exposure to higher inoculum may result in a decreased incubation period for household transmission. Given that the papers compared serial interval to a static estimate of incubation period, the difference in inoculum between household transmission and community transmission may account for the difference between the calculated serial interval and incubation period.

Despite all of these possible sources of error and bias, it is notable that almost all of the studies have calculated serial intervals that fall within the 95% CI of the estimated incubation period as reported by Lauer and colleagues.[60] This would support the conclusion that infectiousness appears to emerge at symptom onset.
Errors in determining viral load distribution before samples collected.

The finding that viral load is highest around the time symptoms are detected in patients suggests that presymptomatic transmission is plausible. However, there is not enough information about the distribution of SARS-CoV-2 viral kinetics in presymptomatic stage to infer when infectiousness begins. Basic assumptions about the distribution will have dramatic effects on our prediction of when infectivity begins, and the specific time between symptom onset and viral load tests can dramatically change our understanding of transmissibility and infectiousness.

Not measuring viral load in presymptomatic stage.

Viral loads appeared at their highest levels when observation in the clinical setting began. Therefore, authors have concluded viral load peaks when symptoms peak. However, without measuring viral load in the presymptomatic phase, the dynamics during the presymptomatic period can only be hypothesized.

Rounding Errors During Calculation of Incubation Period and Serial Intervals.

The datasets from the papers in this review that measured serial interval rounded the date of symptom onset to the nearest day. This is problematic because the difference in serial interval and incubation period calculated in these studies often differed by less than a day. It is therefore difficult to know if the difference between calculated serial interval and incubation period are true differences, or an artefact of rounding error.

Sampling Errors in Nasopharyngeal Swabs.

Nasopharyngeal swabs are an imperfect proxy for viral production. Studies on influenza have shown variability in viral load when sampling left and right nostrils and similar findings will be found in SARS-CoV-2. Any study on viral dynamics must account for high levels of variability in swab samples.
Discussion

This review focused on articles that studied asymptomatic and presymptomatic transmission through case reports, viral kinetics studies, and serial interval calculations. These different approaches have notable shortcomings, which were highlighted in this review. While case studies, in aggregate, can offer compelling insight into the existence of presymptomatic and asymptomatic transmission, these reports have many shortcomings. Even if broad community transmission is not observed, it is still extremely difficult to rule out other sources of infection. Future studies can use viral sequence to better determine sources of infection and transmission chains. Additionally, the temporal variation in what is classified as a symptom of COVID-19, combined with bias and reporting errors, make anecdotal reports of symptom start date unreliable. These factors confound the case reports that highlight asymptomatic or presymptomatic transmission and make it difficult to draw reliable conclusions.

The preliminary SARS-CoV-2 viral dynamics studies demonstrate that viral titer peaks at patient presentation. However, without more knowledge of the temporal distribution of viral load, presymptomatic transmission cannot be conclusively shown. A sharp rise in viral load, as would be observed if viral load followed a Weibull or gamma distribution, may link infectiousness with the start of symptom onset. On the other hand, a normal distribution in viral load would raise the serious possibility of presymptomatic transmission. It is important that the viral dynamics data be validated with culture data on infectivity. As Wolfel and colleagues demonstrated, while viral load is a proxy for infectivity and transmissibility, it is not perfectly correlated.

Nasopharyngeal swabs are an imperfect proxy for viral production, and any study on viral dynamics must account for high levels of variability in swab samples. Future research efforts should focus on other methods of virus harvesting such as throat, blood, fecal, or urine samples, and must prioritize quantifying viral load from individuals in the presymptomatic stage.

The shape of the distribution has the most direct impact on studies attempting to measure serial interval between successive generation of cases. Articles measuring serial interval in this review assumed Weibull, gamma, lognormal, and normal distributions. Differences in the assumption about the distribution of the viral load curves can alter the calculation of how much presymptomatic transmission is occurring. Furthermore, serial interval calculations overrepresent cases of household transmission. It is not possible to deconvolute an observation of shortened serial interval due to presymptomatic transmission from a decreased incubation period due to higher inoculum in household transmission.

Proposed study to characterize presymptomatic transmission

In order to ascertain the temporal viral dynamics and transmissibility of SARS-CoV-2, it is important to study a representative healthy population before, during, and after SARS-CoV-2 infection. It is essential to combine RT-RCR data with viral culturing data to ascertain transmissibility. In particular, such a study would clarify when viral load and transmissibility commence relative to the time of infection, and peak relative to the onset of symptoms. In addition, it can provide insight into the relationship between viral load or Ct and the severity of symptoms.

This study needs to involve a sufficient number of volunteers tested at frequent intervals to obtain a clear answer. Samples need to be collected in a consistent manner using the most reliable available tests. Because of the logistics of such a study, it would be valuable to collect additional information regarding subject demographic features as well as biochemical, immunological, and genetic markers that may be predictive of viral dynamics and transmissibility. Among infected individuals, the additional
determination of viral genomic sequences would allow for molecular epidemiological analysis of transmission between specific individuals.

One way to accelerate the determination of viral kinetics is to focus on a population with high risk of infection and low risk of complications, such as workers in factory at the start of an outbreak, or individuals identified through contact tracing. This population would be ideal to study because these individuals would likely not seek treatment for SARS-CoV-2 infection, therefore the viral dynamics data would not be confounded by therapeutic interventions like antiviral therapy.

While many of the research studies highlighted in this review have supported presymptomatic and asymptomatic transmission, these studies have been inadequate to ascertain the contribution of presymptomatic and asymptomatic transmission in the spread of SARS-CoV-2 infection. Understanding the temporal dynamics of SARS-CoV-2 transmission from asymptomatic and presymptomatic individuals is critical to our efforts to formulate effective and efficient policies to curtail the pandemic.

Acknowledgments:
A special thanks to Dr. Reuben Granich, Dr. Wendy Max, and Jessie Yeung for useful discussion, feedback, and support on this manuscript.
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