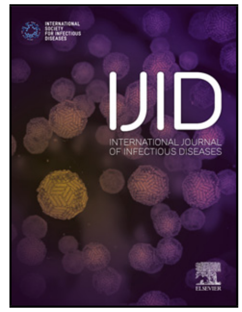

Asymptomatic SARS Coronavirus 2 infection: Invisible yet invincible

Lea A Nikolai, Christian G. Meyer, Peter G. Kremsner, Thirumalaisamy P. Velavan

PII: S1201-9712(20)30706-2

DOI: https://doi.org/10.1016/j.ijid.2020.08.076

Reference: IJID 4573

To appear in: *International Journal of Infectious Diseases*

Received Date: 7 July 2020

Revised Date: 26 August 2020

Accepted Date: 28 August 2020

Asymptomatic SARS Coronavirus 2 infection: Invisible yet invincible

Lea A Nikolai[1], Christian G. Meyer[1,2,3], Peter G. Kremsner[1,4], Thirumalaisamy P. Velavan[1,2,3*]

Authors Affiliations

[1] Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany

[2] Vietnamese-German Center for Medical Research, Hanoi, Vietnam

[3] Faculty of Medicine, Duy Tan University, Da Nang, Vietnam

[4] Centre de Recherches Medicales de Lambarene, Gabon

***Correspondence**

Prof. Dr. Thirumalaisamy P. Velavan

Institute of Tropical Medicine

University of Tübingen

Wilhelmstrasse 27

72074 Tübingen

Germany

Email: velavan@medizin.uni-tuebingen.de

Electronic word count: 2634

Abstract word count: 127

References: 62

Key points: 100 words

Highlights

- Studies confirm the existence of transmission by asymptomatic individuals.
- Characteristics of asymptomatic and presymptomatic infection are not identical.
- Younger age correlates strongly with asymptomatic and mild infections.
- The estimated proportion of asymptomatic infections ranges from 18% to 81%.
- Asymptomatic infections does not provide clear guidance for public-health measures.
- Asymptomatic cases should be reported in official COVID-19 statistics.

- Asymptomatic individuals carrying SARS-CoV-2 are hidden drivers of the pandemic.

Abstract

While successful containment measures of COVID-19 in China and many European countries have led to flattened curves, case numbers are rising dramatically in other countries, with the emergence of a second wave expected. Asymptomatic individuals carrying SARS-CoV-2 are hidden drivers of the pandemic, and infectivity studies confirm the existence of transmission by asymptomatic individuals. The data addressed here show that characteristics of asymptomatic and presymptomatic infection are not identical. Younger age correlates strongly with asymptomatic and mild infections, and children as hidden drivers. The estimated proportion of asymptomatic infections ranges from 18% to 81%. The current perception of asymptomatic infections does not provide clear guidance for public-health measures. Asymptomatic infections will be a key contributor in COVID-19 spread. Asymptomatic cases should be reported in official COVID-19 statistics.

Key words: asymptomatic, presymptomatic, case definition, COVID-19, SARS-CoV-2, herd immunity

Introduction:

Transmission of SARS-CoV-2, the agent causing COVID-19, is driven by virus-containing droplets released from the upper airways and by aerosols that can float, dependent on the airflow, for a prolonged period of time in the environment[1]. Aerosols can be spread by just breathing, while droplets originate from speaking, shouting, sneezing and coughing as well as from singing and playing wind instruments[2-4]. Of great importance are super-spreading events, which are infection clusters and constitute effective chains of SARS-CoV-2 transmission. For instance, such superspreading events have been observed in Hong Kong[5], at religious mass events in Iran[6] and choir rehearsals in the US[7], where singing contributed to a maximum emission of droplets and aerosols and, thus, to successful transmission[4].

Defining "asymptomatic"

Transmission of the virus by infected, albeit asymptomatic individuals has been reported since the early stages of the outbreak[8,9] posing substantial challenges to COVID-19 containment. Spread of COVID-19 likely occurs to a large extent through asymptomatic individuals, as these do not present at health care or testing facilities. Uncertainty about the significance of asymptomatic infections is reinforced by the vagueness with which the term "asymptomatic" is used. WHO defines an asymptomatic case as a laboratory-confirmed infected person without overt symptoms[10]. It remains to be established how thoroughly such a person needs to be examined clinically. Moreover, a distinction between asymptomatic and presymptomatic individuals is often neglected in COVID-19 case definitions.

A distinction between asymptomatic and presymptomatic stages can currently only be made retrospectively, after the occurrence or non-occurrence of clinical symptoms. Recent evidence suggests that elevated serum/plasma lactate dehydrogenase levels may, already in early stages, be indicative of presymptomatic infections and, thus, facilitate early differentiation[11]. Diagnostic imaging cannot distinguish between the two infection stages, as, surprisingly, 30% of asymptomatic individuals showed ground-glass opacities and 27% had diffuse consolidations[12].

Pandemic driven by asymptomatic infections

The frequency and infectivity of asymptomatically infected persons is one of the main reasons why COVID-19 has become a pandemic. Evidence has pointed to the need for strict tracking and testing of all contacts, regardless of apparent symptoms[2,11,13,14]. However, detection of COVID-19 has long been driven by testing patients only, a practice still recommended in the EU/EEA (except Germany) and UK[15]. Meanwhile, some countries have started to extend testing and Luxembourg and the state of Bavaria (Germany) have announced that the entire population of 600,000 and 13 million, respectively, shall be tested to prevent a second wave.

The Centre for Disease Control (CDC) of USA recommends diagnostic tests for both symptomatic and asymptomatic individuals with known or suspected exposure to COVID-19[16], while the European Centre for Disease Control (ECDC) proposes tests mainly for asymptomatic healthcare workers when testing facilities are underutilized[17].

Since 1 April, China has responded to the emerging significance of asymptomatic infections by establishing a separate category of "asymptomatic cases" in its daily COVID 19 statistics[2]. Mathematical modelling clearly supports broader test strategies. A simulation applying data from the Jiangsu Province, China, compared epidemiological data with an estimated asymptomatic proportion of 44% and found that asymptomatic individuals can cause faster and larger outbreaks compared to imported cases[18]. Another analysis indicated that 30% of asymptomatics and 50% of symptomatic patients must be isolated to achieve disease control[19].

Asymptomatic infections during the pandemic

Asymptomatic infections have increasingly been recognized in family clusters with unknown index cases. The first study cluster comprised of five family members from Anyang, China, who developed COVID-19 symptoms and tested positive by RT-PCR after acquiring the infection from the index case, an asymptomatic visitor from Wuhan who later tested positive[20]. Another study with five family members from Luzhou, China, described a patient who developed severe COVID-19 pneumonia after attending a family reunion. Apparently, he had acquired COVID-19 from an asymptomatic relative from Wuhan[21].

Several studies have focused on determining the incidences of asymptomatic infections. On the cruise ship Diamond Princess with 3711 passengers, a major outbreak with 634 cases occurred after an infected asymptomatic passenger had boarded in Hong Kong. Due to the dense living conditions and frequent passenger contacts the R0 value was initially four times higher than in Wuhan[22]. The true asymptomatic proportion, defined as those who never developed symptoms, among all infected passengers was 18%[23]. Similar to the Diamond Princess, another study of an Argentinian expedition cruise ship found that 59% of the 217 passengers tested positive for COVID-19; 81% of those infected were asymptomatic virus carriers[24]. In Vo, the first Italian city with a confirmed COVID-19 fatality, the 3711 was surveyed twice. 2.6 % of the population tested positive before the lockdown and 1.2 % tested positive after the lockdown. Of these, 41% and 45% were asymptomatic before and after the lockdown, respectively[25]. When screening individuals in Gangelt, Germany, 22% of individuals positive for COVID-19 remained asymptomatic[26]. In another study of individuals re-patriated from Wuhan to Japan, 13 of the of 565 (2%) evacuated tested positive and 31% of those evacuated remained asymptomatic after a sufficiently long time to complete the incubation period[27]. A first nationwide population-based study from Spain including 61000 participants from 35883 households concluded that one in three infections seems to be asymptomatic and emphasizes the need for maintaining public health measures, to avoid a second epidemic wave[28].

As these findings differ greatly it is difficult to accurately determine the extent of asymptomatic infections. Discrepancies could result from imprecise definitions of the term "asymptomatic" or a differing understanding of "asymptomatic" in the various studies (Table1). Extremely high incidences could result from unintended inclusion of presymptomatic and very mild cases. The true incidence of asymptomatic infections can only be determined if close surveillance is installed and continued at least over the estimated average incubation period of at least 5 days in order not to miss a possible onset of symptoms.

Asymptomatic Infectivity: viral load and viral shedding

When assessing public health risks raised by asymptomatic COVID-19 cases it is important to determine whether the infectivity varies between asymptomatic, presymptomatic and symptomatic individuals. A study of the first 243 patients in Singapore revealed a proportion of 6% presymptomatic cases with transmission occurring 1-3 days before the onset of symptoms[14]. Data from three Chinese hospitals, including 24 asymptomatic subjects, showed an average SARS-CoV-2 carrier period of 22 days. The time from exposure to eventual negativity indicates that asymptomatically infected persons likely carry the virus for a rather long period[9]. Infectivity exists in presymptomatic and/or asymptomatic men; however, further analyses of the viral loads and the duration of viral shedding are required. Viral shedding is the release of the virus from somatic cells after replication; it does not necessarily imply that the virus is infectious[29]. The virus load is measured by the cycle threshold (Ct value), which corresponds to the amplification cycles in the diagnostic RT-PCR assays. Ct values increase with decreasing viral load and low Ct values indicate a high viral load. In addition, the duration of virus detectability serves as an indicator of infectivity.

In a study with 21 infected Chinese, a subgroup of five asymptomatic patients had the longest period of RNA positivity with prolonged viral shedding. The virus was thriving in asymptomatic subjects for a median of 18 days compared to mild (median 10 days) and severe COVID-19 cases (median 14 days)[30]. Another study from Wanzhou district, China, compared 37 asymptomatic with 37 symptomatic individuals. Although the initial Ct values were similar, asymptomatic individuals showed prolonged virus release (asymptomatic individuals: median 19 days, symptomatic individuals 14 days)[12]. Of interest is also another study of 18 patients from Zhuhai, China, where viral loads in asymptomatics were similar to those in patients[31]. In the study from the Vo community in Italy, no significant differences were found between viral loads of asymptomatic and presymptomatic individuals and no differences in the duration of virus detectability was seen[25].

Data of 78 infected subjects from Wuhan identified 33 (42%) asymptomatic infections with shorter periods of viral shedding compared to symptomatic patients (8 vs.19 days)[32]. A cohort of 71 South Koreans included 3 presymptomatic and 10 asymptomatic subjects. Asymptomatics had lower Ct values than presymptomatic subjects over a period of 15 days, indicating a higher viral load[33]. Of 31 infected persons from Guangzhou, China, who were tested positive 22 proved to be presymptomatic on admission and developed symptoms later, whereas 9 remained asymptomatic. There was a statistically significant difference between asymptomatic and presymptomatic infection, with higher Ct values in asymptomatic individuals than in presymptomatics. However, there was no significant difference in viral shedding[34]. In addition, infectivity was found to be highest roughly a day before symptom onset and it was estimated that 44% of secondary cases were infected by a presymptomatic carrier[35].

In order to ascertain an asymptomatic, who did not meet the case definition, the detection of SARS-CoV-2 by RT-PCR on nasopharyngeal and oropharyngeal swabs is the only currently available standard diagnosis[36]. Recent studies raise the question of the reliability of RT-PCR based on increasing evidence of false negative cases and concerns about its applicability[37,38]. False-negative results can have a significant negative impact on efforts to contain the epidemic. The probability of false negative results in RT-PCR tests is influenced by the time since exposure and the onset of symptoms[39]. On the other hand, technical problems, insufficient virus load and inadequate and inappropriate sampling may be other reasonable causes of false negative results. The challenges in laboratory diagnosis of SARS-CoV-2 by RT-PCR is multifactorial and well-defined guidelines are warranted to fill the gaps in detection of SARS-CoV-2 in asymptomatics.

Age stratified asymptomatic infection

When analysing common characteristics of patients, young age often correlated with asymptomatic or mild manifestations of COVID-19. Among 78 patients from Wuhan, China, asymptomatic individuals were younger than symptomatic patients (median age 37 vs. 56 years)[32]. In Nanjing, China, of 24 initially asymptomatic subjects, 29% who never showed symptoms were significantly younger than the presymptomatic group[40]. These results are supported by the data from the cruise liner Diamond Princess, where of 96 asymptomatic persons 11 later developed symptoms that made them presymptomatic. The probability of turning to a presymptomatic stage increased with age[41].

In fact, a much lower prevalence of COVID-19 is observed in children than in adults, with people under 18 years accounting for 5% of cases only in the USA compared to 22% in the total population[42]. Of 1412 Chinese children with infection, 4% and 51% were categorized as asymptomatic and mild, respectively[43].

Recent evidence suggests that the entry of SARS-CoV-2 via the ACE2 receptor is facilitated by the membrane-bound serine protease TMPRSS2, which primes the viral S protein for fusogenic activity[44,45]. Since *TMPRSS2* is a gene that associates with androgen levels, a higher expression occurs in males, which provides one explanation why they are more likely to develop severe COVID-19[46]. This association also applies to the distinction between preadolescents and adults and is in line with low incidences and rather mild disease courses in children[47]. Since this also indicates a higher incidence of asymptomatic infections in younger people, it needs to be examined whether this group, especially children, could silently, yet efficiently, contribute to the spread of COVID-19.

In Geneva, Switzerland, 79% of all RT-PCR-positive children under 16 years of age were infected in household clusters[48] and in Wuhan, China, as much as 90% of this group were infected by a family member[49]. A systematic review identified 31 household clusters, of which only 10% had paediatric index cases compared to 54% in H5N1 influenza[50]. These observations, together with evidence of lower viral loads and milder respiratory symptoms in children, have led to the conclusion that children are unlikely to be the main cause of the pandemic[51]. Consequently, re-opening of kindergartens and schools has been proposed. A low attack rate among children may yet be biased, because the risk of infection is lowest for children[52]. Current data from Berlin, Germany, did not show significant differences in viral loads between age groups, suggesting that children may be as infectious as adults[53].

Neglecting the role of children in the spread of COVID-19 is precarious. It is important in modelling the pandemic to undertake careful surveillance, including asymptomatic children with rates of infections assessed by serology in order to better characterize childhood infection and the role of children in transmission networks[54]. Children need protection, as some become ill, although severely only in the very minority of cases. COVID-19 vaccination in children may provide protection for older, unvaccinated populations. Vaccination in childhood will lead to a great deal of immunity required for overall protection in any population[54,55].

Asymptomatic infection: Hope for herd immunity

Studies suggesting high incidences of transmission through asymptomatic individuals have raised hope that broad immunization of the population occurs unnoticeably. In general, about two thirds of a population must be immunized to achieve herd immunity. On 24 June 2020, the countries with highest incidences of COVID-19 were the USA, Brazil, Russia, India and the United Kingdom, with case numbers representing 0.71%, 0.54%, 0.43%, 0.03% and 0.47% of the population, respectively, thus being far from herd immunity[56].

In Gangelt (Germany), an event linked to carnival celebrations caused SARS-CoV-2 spread throughout the city and resulted in 3% of the population with positive RT-PCR results. Serological screening revealed later that 16% of the population were exposed[26]. Even in densely populated and severely affected areas the prevalence of anti-SARS-CoV-2 antibodies is still relatively low, e.g. 11% in Madrid, Spain[57], 15% in London, UK [58] and 20% in New York City, USA[59].

It is still unclear whether asymptomatic infections lead to protective immunity. It was observed that, although all patients with severe and mild COVID-19 experienced seroconversion during or after hospitalization, only 1 in 5 asymptomatic patients seroconverted[30]. Another comparison between an asymptomatic and a symptomatic cohort showed that IgG levels were significantly higher in symptomatic group[12]. However, data from two hospitals in Hong Kong suggest that the severity of the disease is not correlated with serum antibody levels[60]. It would not only be misleading, but dangerous to rely on silent immunization.

Apparently, so far only a small proportion of the population has been exposed to SARS-CoV-2.

Public health implications

The current perception of asymptomatic infections does not provide clear guidance for public health measures. As asymptomatic and presymptomatic infections are not distinguishable on a first sight, they may pose a significant threat to public health during the unlocking lockdown strategies currently implemented in many countries.

Therefore, public health measures need to further–mandatorily and for an unforeseeable period of time – include sound hygiene measures and personal protective equipment to prevent spread by asymptomatic individuals. Contacts of infected persons must test for COVID-19, regardless of symptoms. Asymptomatic cases should be reported separately in official COVID-19 statistics and shifts from asymptomatic to symptomatic stages must be reported to health authorities. Mass rallies and major events need further be postponed or cancelled.

Conclusions

Asymptomatic infections are an important aspect of SARS-CoV-2 infection and the data addressed here show that the characteristics of asymptomatic and presymptomatic infection are not identical. Asymptomatic infections will be a key contributor in COVID-19 spread. Infectivity studies confirm the existence of transmission by asymptomatic individuals but are contradictory when comparing viral loads and virus shedding in symptomatic and asymptomatic infections. Younger age correlates strongly with asymptomatic and mild infections and therefore suggests children as hidden drivers of the pandemic. However, since childhood infections are usually far below the age average in COVID-19 infections, the role of children in transmission events is not yet clear.

While the public health measures might be practicable in wealthy countries with well-established and rather stable health care systems, the question on how the pandemic will affect low- and middle-income countries as observed in South America or on the African continent remains still unresponded[61,62]. The international community is obliged to pay attention to the spread of COVID-19 to low-income countries, as health systems could become severely overburdened and the pandemic could continue to elude control, hitting those hardest with the least protection.

Conflict of interest: All authors disclose no conflict of interest.

Funding source: The authors acknowledge the Federal Ministry of Education and Research (BMBF) (BMBF-01KI2052) and the Federal Ministry of Health (BMG) (BMG-ZMVI1-1520COR801).

Ethical Approval: Not applicable

Contribution statement: All authors have an academic interest and contributed equally. The authors TPV and LAN conducted literature search, collected data and collated all information and wrote the review. The authors CGM and PGK contributed to the study design and revised the draft review. TPV is a member of the Pan African Network for Rapid Research, Response, and Preparedness for Infectious Diseases Epidemics consortium (PANDORA-ID-NET RIA2016E-1609).

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

All authors disclose no conflict of interest. All authors have an academic interest and contributed equally. TPV is a member of the Pan African Network for Rapid Research, Response, and Preparedness for Infectious Diseases Epidemics consortium (PANDORA-ID-NET RIA2016E-1609).

References:

1. Meselson M. Droplets and Aerosols in the Transmission of SARS-CoV-2. *N Engl J Med.* 2020;382(21):2063.
2. Zhang J, Wu S, Xu L. Asymptomatic carriers of COVID-19 as a concern for disease prevention and control: more testing, more follow-up. *Biosci Trends.* 2020.
3. Zhen-Dong Y, Gao-Jun Z, Run-Ming J, et al. Clinical and transmission dynamics characteristics of 406 children with coronavirus disease 2019 in China: A review. *J Infect.* 2020.
4. Asadi S, Wexler AS, Cappa CD, Barreda S, Bouvier NM, Ristenpart WD. Aerosol emission and superemission during human speech increase with voice loudness. *Sci Rep.* 2019;9(1):2348.
5. Adam D WP, Wong J, Lau E, Tsang T, Cauchemez S, Leung G, Cowling B. Clustering and superspreading potential of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections in Hong Kong. In. *Reserach Square*2020.
6. Mubarak N, Zin CS. Religious tourism and mass religious gatherings - The potential link in the spread of COVID-19. Current perspective and future implications. *Travel Med Infect Dis.* 2020;36:101786.
7. Hamner L, Dubbel P, Capron I, et al. High SARS-CoV-2 Attack Rate Following Exposure at a Choir Practice - Skagit County, Washington, March 2020. *MMWR Morb Mortal Wkly Rep.* 2020;69(19):606-610.
8. Pan X, Chen D, Xia Y, et al. Asymptomatic cases in a family cluster with SARS-CoV-2 infection. *Lancet Infect Dis.* 2020;20(4):410-411.
9. Yan X, Han X, Fan Y, Fang Z, Long D, Zhu Y. Duration of SARS-CoV-2 viral RNA in asymptomatic carriers. *Crit Care.* 2020;24(1):245.
10. WHO. *Clinical management of COVID-19: interim guidance.* 27 May 2020 2020.
11. Ooi EE, Low JG. Asymptomatic SARS-CoV-2 infection. *Lancet Infect Dis.* 2020.
12. Long QX, Tang XJ, Shi QL, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med.* 2020.
13. Baggett TP, Keyes H, Sporn N, Gaeta JM. COVID-19 outbreak at a large homeless shelter in Boston: Implications for universal testing. *medRxiv.* 2020:2020.2004.2012.20059618.
14. Wei WE, Li Z, Chiew CJ, Yong SE, Toh MP, Lee VJ. Presymptomatic Transmission of SARS-CoV-2 - Singapore, January 23-March 16, 2020. *MMWR Morb Mortal Wkly Rep.* 2020;69(14):411-415.
15. *European Centre for Disease Prevention and Control. Strategies for the surveillance of COVID-19. Stockholm: ECDC; 2020.* European Centre for Disease Prevention and Control; 09.April 2020.
16. Prevention CfDCa. Overview of Testing for SARS-CoV-2. https://www.cdc.gov/coronavirus/2019-ncov/hcp/testing-overview.html. Published 2020. Accessed June 23, 2020.
17. Control ECfDPa. Testing strategies. https://www.ecdc.europa.eu/en/covid-19/surveillance/testing-strategies. Published 2020. Accessed June 23, 2020.
18. Sun T, Weng D. Estimating the effects of asymptomatic and imported patients on COVID-19 epidemic using mathematical modeling. *J Med Virol.* 2020.
19. Kassa SM, Njagarah JBH, Terefe YA. Analysis of the mitigation strategies for COVID-19: From mathematical modelling perspective. *Chaos Solitons Fractals.* 2020;138:109968.
20. Bai Y, Yao L, Wei T, et al. Presumed Asymptomatic Carrier Transmission of COVID-19. *Jama.* 2020;323(14):1406-1407.
21. Ye F, Xu S, Rong Z, et al. Delivery of infection from asymptomatic carriers of COVID-19 in a familial cluster. *Int J Infect Dis.* 2020;94:133-138.
22. Rocklöv J, Sjödin H, Wilder-Smith A. COVID-19 outbreak on the Diamond Princess cruise ship: estimating the epidemic potential and effectiveness of public health countermeasures. *J Travel Med.* 2020;27(3).

23. Mizumoto K, Kagaya K, Zarebski A, Chowell G. Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020. *Euro Surveill.* 2020;25(10).
24. Ing AJ, Cocks C, Green JP. COVID-19: in the footsteps of Ernest Shackleton. *Thorax.* 2020.
25. Lavezzo E, Franchin E, Ciavarella C, et al. Suppression of COVID-19 outbreak in the municipality of Vo, Italy. *medRxiv.* 2020:2020.2004.2017.20053157.
26. Streeck H, Schulte B, Kuemmerer B, et al. Infection fatality rate of SARS-CoV-2 infection in a German community with a super-spreading event. *medRxiv.* 2020:2020.2005.2004.20090076.
27. Nishiura H, Kobayashi T, Miyama T, et al. Estimation of the asymptomatic ratio of novel coronavirus infections (COVID-19). *Int J Infect Dis.* 2020;94:154-155.
28. Pollán M P-GB, Pastor-Barriuso R, Oteo J, Hernán MA, Pérez-OlmedaM,, Sanmartín JL F-GA, Cruz I, Fernández de Larrea N, Molina M,, Rodríguez-Cabrera F MM, Merino-Amador P, Paniagua JL,, Muñoz-Montalvo JF BF, Yotti R, ENE-COVID Study group. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. *THE LANCET.* 2020.
29. Atkinson B, Petersen E. SARS-CoV-2 shedding and infectivity. *Lancet.* 2020;395(10233):1339-1340.
30. Yongchen Z, Shen H, Wang X, et al. Different longitudinal patterns of nucleic acid and serology testing results based on disease severity of COVID-19 patients. *Emerg Microbes Infect.* 2020;9(1):833-836.
31. Zou L, Ruan F, Huang M, et al. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. *N Engl J Med.* 2020;382(12):1177-1179.
32. Yang R, Gui X, Xiong Y. Comparison of Clinical Characteristics of Patients with Asymptomatic vs Symptomatic Coronavirus Disease 2019 in Wuhan, China. *JAMA Netw Open.* 2020;3(5):e2010182.
33. Kim SE, Jeong HS, Yu Y, et al. Viral kinetics of SARS-CoV-2 in asymptomatic carriers and presymptomatic patients. *Int J Infect Dis.* 2020;95:441-443.
34. Zhou R, Li F, Chen F, et al. Viral dynamics in asymptomatic patients with COVID-19. *Int J Infect Dis.* 2020;96:288-290.
35. He X, Lau EHY, Wu P, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nat Med.* 2020;26(5):672-675.
36. Younes N, Al-Sadeq DW, Al-Jighefee H, et al. Challenges in Laboratory Diagnosis of the Novel Coronavirus SARS-CoV-2. *Viruses.* 2020;12(6).
37. Arevalo-Rodriguez I, Buitrago-Garcia D, Simancas-Racines D, et al. FALSE-NEGATIVE RESULTS OF INITIAL RT-PCR ASSAYS FOR COVID-19: A SYSTEMATIC REVIEW. *medRxiv.* 2020:2020.2004.2016.20066787.
38. Piras A, Rizzo D, Uzzau S, De Riu G, Rubino S, Bussu F. Inappropriate Nasopharyngeal Sampling for SARS-CoV-2 Detection Is a Relevant Cause of False-Negative Reports. *Otolaryngol Head Neck Surg.* 2020:194599820931793.
39. Kucirka LM, Lauer SA, Laeyendecker O, Boon D, Lessler J. Variation in False-Negative Rate of Reverse Transcriptase Polymerase Chain Reaction-Based SARS-CoV-2 Tests by Time Since Exposure. *Ann Intern Med.* 2020;173(4):262-267.
40. Hu Z, Song C, Xu C, et al. Clinical characteristics of 24 asymptomatic infections with COVID-19 screened among close contacts in Nanjing, China. *Sci China Life Sci.* 2020;63(5):706-711.
41. Sakurai A, Sasaki T, Kato S, et al. Natural History of Asymptomatic SARS-CoV-2 Infection. *N Engl J Med.* 2020.
42. Prevention CfDCa. Cases in the U.S. https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/cases-in-us.html. Published 2020. Accessed June 24, 2020.
43. Dong Y, Mo X, Hu Y, et al. Epidemiology of COVID-19 Among Children in China. *Pediatrics.* 2020;145(6).
44. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell.* 2020;181(2):271-280.e278.

45. Zang R, Gomez Castro MF, McCune BT, et al. TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. *Sci Immunol.* 2020;5(47).
46. Bhowmick NA, Oft J, Dorff T, et al. COVID-19 and androgen targeted therapy for prostate cancer patients. *Endocr Relat Cancer.* 2020.
47. Wambier CG, Goren A, Vaño-Galván S, et al. Androgen sensitivity gateway to COVID-19 disease severity. *Drug Dev Res.* 2020.
48. Posfay-Barbe KM, Wagner N, Gauthey M, et al. COVID-19 in Children and the Dynamics of Infection in Families. *Pediatrics.* 2020.
49. Lu X, Zhang L, Du H, et al. SARS-CoV-2 Infection in Children. *N Engl J Med.* 2020;382(17):1663-1665.
50. Zhu Y, Bloxham CJ, Hulme KD, et al. Children are unlikely to have been the primary source of household SARS-CoV-2 infections. *medRxiv.* 2020:2020.2003.2026.20044826.
51. Ludvigsson JF. Children are unlikely to be the main drivers of the COVID-19 pandemic - A systematic review. *Acta Paediatr.* 2020.
52. García-Salido A. SARS-COV-2 children transmission: the evidence is that today we do not have enough evidence. *Acta Paediatr.* 2020.
53. Jones TC, Mühlemann B, Veith T, et al. An analysis of SARS-CoV-2 viral load by patient age. *medRxiv.* 2020:2020.2006.2008.20125484.
54. Velavan TP, Pollard AJ, Kremsner PG. Herd Immunity and Vaccination of children for COVID19. *Int J Infect Dis.* 2020.
55. Kao CM, Orenstein WA, Anderson EJ. The Importance of Advancing SARS-CoV-2 Vaccines in Children. *Clin Infect Dis.* 2020.
56. Johns Hopkins University & Medicine. COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU). https://coronavirus.jhu.edu/map.html. Published 2020. Accessed June 24, 2020.
57. Gobierno de España MdCeI, Ministerio de Sanidad, Instituto de Salud Carlos III, Consejo Interterritorial. Estudio ENE-Covid19: Primera Ronda Estudio Nacional de sero-Epidemiología de la infección por SARS-CoV-2 en España (ENE-Covid19) Informe Preliminar 13 de Mayo de 2020. https://www.lamoncloa.gob.es/serviciosdeprensa/notasprensa/sanidad14/Documents/2020/130520-ENE-COVID_Informe1.pdf. Published 2020. Accessed June 26, 2020.
58. England PH. Weekly Coronavirus Disease 2019 (COVID-19) Surveillance Report Summary of COVID-19 surveillance systems. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/888254/COVID19_Epidemiological_Summary_w22_Final.pdf. Published 2020. Accessed June 26, 2020.
59. State NY. Amid Ongoing COVID-19 Pandemic, Governor Cuomo Announces Results of Completed Antibody Testing Study of 15,000 People Showing 12.3 Percent of Population Has COVID-19 Antibodies. https://www.governor.ny.gov/news/amid-ongoing-covid-19-pandemic-governor-cuomo-announces-results-completed-antibody-testing. Published 2020. Accessed June 24, 2020.
60. To KK, Tsang OT, Leung WS, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis.* 2020;20(5):565-574.
61. Ntoumi F, Velavan TP. COVID-19 in Africa: between hope and reality. *Lancet Infect Dis.* 2020.
62. Wang J, Xu C, Wong YK, et al. Preparedness is essential for malaria-endemic regions during the COVID-19 pandemic. *Lancet.* 2020;395(10230):1094-1096.

Table 1: Limited overview on reported studies on asymptomatic infection among adults and children

Reported studies	Sample size	Country	Esti-mates	Refer-ences
Passenger ship: Diamond Princess	n=3711	Yokohama, Japan	18%	[22, 23]
Argentinian expedition cruise ship	n=217	Montevideo, Uru-guay	81%	[24]
Two-point prevalence survey	n=2343	Vo, Padua, Italy	41% and 45%	[25]
Sero-epidemiological study	n=919	Gangelt, Germany	22%	[26]
Repatriated passengers	n=565	Japan	31%	[27]
Nationwide population-based study	n=61000	Spain	22 to 36%	[28]
Presymptomatic surveillance study	n=243	Singapore	6%	[14]
Followed up case series	n=78	Wuhan, China	42%	[32]
Viral dynamics: Asymptomatic patients	n=31	Guangzhou, China	29%	[34]
Nationwide case series: Children	n= 2135	China	4%	[43]