Emerging Evidence on COVID-19

Evidence Brief on viral load and the likelihood of transmission during the infectious period of SARS-CoV-2

Introduction

What is the relationship between viral load and the likelihood of transmission during the infectious period of SARS-CoV-2? Does this vary by presence of symptoms, severity of symptoms, risk factors (e.g., age, chronic health conditions) or by infection with the currently circulating SARS-CoV-2 variants of concern?

The correlation between viral load and likelihood of transmission has been studied throughout the pandemic in order to characterize when during an infection with SARS-CoV-2 a person may be more infectious and most likely to transmit the virus. Research has also attempted to characterize the attributes of individuals or characteristics of infection that may be associated with higher risk of transmission. During Winter 2021 new research has indicated that variants of concern (e.g., B.1.1.7, B.1.351, and P.1) may have different transmission dynamics and virulence than wild-type strains that were circulating earlier in the pandemic. This could be due to a multitude of interlacing factors (e.g., longer persistence of viral RNA shedding, decreased immune response, or lower binding energy between the SARS-CoV-2 spike protein and human receptors). The current evidence base is conflicting on whether variants of concern have higher viral loads. Some studies have reported higher viral loads in variant cases (B.1.1.7 and P.1) compared to wild-type (1-5), while three new studies reported no significant difference (6-8). Further investigations are necessary to determine what factors are contributing to the increased transmission observed with the circulating variants of concern. On the other hand, recent vaccination studies indicate that vaccination may prevent against symptomatic infection and may reduce viral load by 1.6 times to 20 times in infections occurring after the first dose of vaccine (9-11). Thus, even if vaccines do not fully prevent infection, reducing viral load during infection will also likely reduce transmission. This evidence brief focuses on research that investigates the relationship between viral load and likelihood of transmission during the infectious period of SARS-CoV-2 published up to March 31, 2021.

The most common proxy measurement of SARS-CoV-2 viral load is the cycle threshold (Ct) value, which is the number of cycles during reverse transcription polymerase chain reaction (RT-PCR) required to reach a threshold of detection for a certain gene target. Within this model, lower Ct values indicate a higher viral load and where provided, an estimate of viral load, copies/mL, can be calculated. The calculation of copies/mL requires a standardization process based on the amplification target in the extract and the Ct value. There are several downsides to using Ct values as a proxy of viral load. First, amplification efficiency is influenced by the assay itself and factors associated with sample collection (e.g., amount of sample material collected) (12, 13). Studies are often highly heterogeneous in the sampling methods, detection assay, and gene targets used. Second, the Ct value upper bound cut-off that determines a positive PCR result has been inconsistent among

studies, though most reported positive values at Ct ≤35. It is recommended that a standard curve using reference materials or in-house plasmid controls with known viral copy numbers be utilized to interpret Ct values as viral loads – this would allow for appropriate quantification (e.g., copies/mL) (14). However, there has been wide heterogeneity and inconsistency of the standard curves calculated across studies, so precaution is necessary when interpreting viral load results in the COVID-19 literature.

Detection of viral RNA by RT-PCR does not provide proof of infectivity as this test also gives positive results when non-infectious virus particles are present. Recovery of replication-competent virus has been used as a measure of infectiousness (i.e., transmission potential). This is most often accomplished using cell culture. The detection of subgenomic RNA has also been recommended as a potential proxy for shedding of replication-competent virus (15), although consensus on this application is lacking (16). While viral RNA shedding is often observed in respiratory samples collected more than 15-17 days post symptom onset (17), replicative virus has in most instances not been isolated past 10 days in mild cases (18-22). The correlation of SARS-CoV-2 viral loads and Ct values with isolation of replicative virus is an important topic of interest when investigating transmission potential and is explored in this review.

Key Points

- The evidence brief identified 27 studies including 2 systematic reviews, 5 prospective cohorts, 5 retrospective cohorts, 1 case-control, 7 cross-sectional studies, 1 surveillance study, 4 case series, 1 contact tracing study, and 2 modelling studies.

- Across all studies, transmission is most likely to occur when, samples contained replicative virus, which occurred when Ct values were low (<30) and the sample was taken less than 8-10 days from symptom onset.

Studies that investigate an association between viral load and evidence of replicative virus (Culture/subgenomic RNA) (n=15):

- Replicative virus was most likely to be isolated from samples with Ct values <30 or viral loads >1x 10^6 copies/mL. Samples with Ct values ≤25 demonstrated replicative virus at a rate >90%.

- The most recently published systematic review on this topic reported significant correlation between Ct value and culture positivity rates. The probability of recovery of virus from specimens with Ct > 35 was 8.3% (95% CI: 2.8% to 18.4%). Further, the odds for culturing replicative virus has been reported to decrease by 0.64 for every one unit increase in Ct (95% CI 0.49 to 0.84, p<0.001).

- Recovery of replicative virus was unlikely in samples collected >8-10 days post symptom onset, even in samples with Ct values <35. Thus, Ct value and days post symptom onset assessed in tandem may be an effective method for deciding the likelihood an individual is still infectious.

- Cases with positive culture identified at greater than 8-10 days or at Ct values >35 were more likely to be severe or immunocompromised cases.

- There was heterogeneity in the sampling and detection methods used across studies. The overall association between Ct value and isolation of replicative virus did not appear to differ depending on SARS-CoV-2 gene target (e.g., Nucleocapsid (N), Envelope (E), Spike protein (S)) used for the PCR test.

Studies that investigate an association between viral load and likelihood of transmission (n=12):

- Modelling studies demonstrated that viral loads peak on average 5 days post exposure and 1-2 days post symptom onset, with a short period (<2 days) of high transmission risk after which the likelihood of transmission quickly diminishes by 7-10 days post symptom onset. Highly infectious cases can shed tens to thousands of SARS-CoV-2 virions/min, especially between 1-5 days post symptom onset.

- Index cases with high viral loads (>10^6 copies/mL or Ct values <30) were more likely to transmit SARS-CoV-2 to household contacts.

- Transmission from index cases with high Ct values (>35) have occurred, but the transmission risk is much lower (8%).

- The majority of secondary household cases were detected within 10 days post symptom onset of the index case.

- High viral loads have been reported during the pre-symptomatic period and up to 8-10 days post symptom onset. It is during this time when Ct values are low (<30) that there is a high probability of transmission.

Overview of the Evidence

A total of 27 studies were included in this review, including several observational studies. Multiple studies are pre-prints and have not undergone a peer-review process. Prospective cohorts are at lower risk of bias than retrospective cohorts and cross-sectional analyses of medical record data or routinely collected surveillance data on COVID-19. Some of these studies appeared to have good generalizability as they represented large or national databases. However, retrospective cohorts and cross-sectional studies are at higher risk of bias due to their retrospective nature, as well as the increased risk of having missing and confounding data. Case series suffer from low sample size, selection bias and recall bias (e.g., self-report symptom onset) and lack of generalizability. Predictive models were also included in this review, and their analyses represent an approximation of a real situation and can be used to compare options or scenarios, keeping in mind the limitations of the models used. Case reports and studies/reviews published prior to the latest relevant systematic reviews on this topic (search conducted up to September 2020) were excluded from this review, as it was expected they would be captured in the systematic reviews (23, 24).

Studies were highly heterogeneous in the designs, sampling methods, detection tests, and gene targets used. Thus, results cannot be directly compared across studies. The RT-PCR test results depend on the quality of the sample as well as the assay used (i.e., the chosen primers, reagents, and gene targeted) which determine the accuracy of the test. These technical differences in how the tests were conducted also made comparisons across studies difficult.

While many studies reported that viral loads differed by symptoms or risk factors, very few actually reported on this relationship in the context of transmission potential. Similarly, evidence to date on the potential relationship between viral load and increased transmissibility of SARS-CoV-2 variants of concern such as B.1.1.7 and P.1 are currently assumed to be related based on surveillance data reporting lower Ct values among infections (1-5) and recent models of rising VOC cases (25). However, the evidence is currently conflicting on whether B.1.1.7 and P.1 do in fact result in higher viral loads than wild-type SARS-CoV-2 (6-8). Evidence on whether other variants of concern, such as B.1.351, might be associated with higher viral loads has not yet been substantiated by primary data. Future empirical research will hopefully help close these knowledge gaps. No studies were identified that directly analyzed how the relationship between viral load and likelihood of transmission varied by infection with the currently circulating SARS-CoV-2 variants of concern. As such, the evidence presented below reflects wild-type strains as opposed to variants of concern.

CONTENTS

ASSOCIATION BETWEEN VIRAL LOAD AND EVIDENCE OF REPLICATIVE VIRUS

- Thirteen studies reported detection of replicative virus via cell culture and three studies reported detection via subgenomic RNA.
- Across all studies, replicative virus was more likely to be isolated from samples with lower Ct values than samples with high Ct values. Detection of replicative virus varied by viral load and timing of collection post symptom onset. No studies reported whether the association between viral load and evidence of replicative virus varied by risk factors (e.g., age, sex) or by infection with SARS-CoV-2 variants of concern.
    - Replicative virus was most likely to be isolated from samples with Ct values <30 (26-34) or viral loads >1x 10^6 copies/mL (23, 35).
        - A systematic review estimated that the probability of recovery of virus from specimens with Ct > 35 was 8.3% (95% CI: 2.8% to 18.4%) (23). Further, the odds for culturing live

virus has been reported to decrease by 0.64 for every one unit increase in Ct (95% CI 0.49 to 0.84, p<0.001) (23).
    - At Ct=25, up to 70% of patients remain positive in culture. At Ct=30 this value drops to 20%. At Ct=35 only <3% of cultures are positive (30).
    - In both mild and severe groups, viral replication was significantly more likely to be detected for samples with lower Ct values (p<0.001) (36). Samples with Ct values ≤ 25 demonstrated replicative virus at a rate >90% (36).
- Recovery of replicative virus was unlikely in samples collected >8-10 days post symptom onset, even in samples with Ct values <35 (34, 37-39). Thus, the probability of isolating replicative virus is highest prior to 8-10 days post symptom onset when Ct levels are low.
- Evidence of replicative virus has been detected in immunocompetent asymptomatic/mild cases with high Ct values (>35) (26, 33, 36), but such instances appear to be rare. In general, cases with positive culture identified at greater than 8-10 days or at Ct values >35 were more likely to be severe or immunocompromised cases (36, 39).

- There was heterogeneity in the sampling and detection methods used across studies.
    - RT-PCR gene targets reported across studies included the SARS-CoV-2 N gene (28, 29, 31, 37), E gene (27, 28, 30, 32, 36), S gene (26, 29), and ORF1ab (29). The overall association between viral load and isolation of replicative virus did not appear to differ depending on gene target.
    - The majority of studies were conducted using nasopharyngeal swabs (26-30, 30, 33-38, 38). However, oropharyngeal (34, 35, 37), sputum (34, 37), bronchial aspirate (36), serum (37), urine (37) and stool (37) swabs were also reported. Culture was more likely to be positive in nasopharyngeal, oropharyngeal, and sputum swabs than other specimen types (37).

Table 1: Studies that investigate association between viral load and evidence of replicative virus (n=15)

STUDY	METHOD	KEY OUTCOMES
Culture studies (n=13)		
Jefferson (2020) (23) Systematic review UK* Dec 2020*	Searched databases for studies attempting to culture or observe SARS-CoV-2 in specimens with RT-PCR positivity published up to September 10, 2020. Twenty-nine studies were captured, ten of which analysed the relationship between Ct values and virus culture.	- 5 studies reported no growth in specimens based on Ct cut-offs >24 to 35. The estimated probability of recovery of virus from specimens with Ct > 35 was 8.3% (95% CI: 2.8% to 18.4%). All donors with Ct > 35 (n=5) producing live culture were symptomatic. - One study reported the odds for culturing live virus decreased by 0.64 for every one unit increase in Ct (95% CI 0.49 to 0.84, p<0.001). - One study reported similar results in line with empirical evidence of an increased Ct of 0.58 per day since symptoms started.

		- One study reported that no successful viral culture was obtained from samples with viral loads less than 1x 10^6 copies/mL. - Viral load and probability of growing live virus of SARS-CoV-2 appears to peak much sooner than that of SARS CoV-1 or MERS-CoV.
Killerby (2021) (37) Prospective cohort study USA Mar 2021*	Nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, sputum (if available), serum, urine and stool specimens were collected every 2–3 days from the first 14 symptomatic patients detected in the USA and tested by rRT-PCR (targeting the N1, N2, or N3 gene). For a sample to be positive, all three gene targets had to be detected. Inconclusive results meant two gene targets were detected. Viral culture (Vero CCL-81 cells) was attempted on rRT-PCR positive and inconclusive specimens (n=131) collected during days 0-29 post illness onset.	- Virus was not recovered from respiratory specimens collected more than 8 days post symptom onset. - Successful culture was observed in 14% (8/57) of NP swabs, 10% (4/92) of OP swabs, and 14% (2/14) of sputum specimens with nucleocapsid (N)1, N2, and N3 Cts ranging from 16.5–32.5, 17.7–32.6, and 16.7–31.4 respectively. - Ct values were all significantly lower ($p<0.0001$ for all 3 gene targets) among specimens from which virus was successfully recovered in culture versus not. - Live virus was not recovered from serum or stool specimens, from inconclusive respiratory specimens, or from specimens collected after symptom resolution, despite continued detection of viral RNA.
Antar (2021) (34) *Preprint* Prospective cohort study USA Apr-July 2020	Outpatients (n=95) self-collected mid-turbinate nasal, oropharyngeal (OP), and oral fluid a median of 6 times over 1-3 months. Samples were tested for viral RNA using the RT-PCR Abbott m2000 platform targeting. Ct values < 31.5 were considered positive. Positive nasal-OP samples by RT-PCR were tested for propagation of SARS-CoV-2 in cell culture (methods not described). Also analyzed whether oral fluid anti-SARS-CoV-2 IgG could be used to predict which	- No samples collected more than 11 days post symptom onset tested positive for viral culture, even in an immunocompromised case who had positive RT-PCRs with low Ct values two months post symptom onset. - Virus culture was positive only in samples with Ct values <17. - Mean Ct values were all significantly lower among specimens from which virus was successfully recovered in culture versus culture negative samples ($p<0.00001$). - 14/15 positive for oral fluid anti-S-RBD IgG, with Ct values <20, were negative for virus culture. The one culture positive sample was collected on day 11 post symptom onset, which is around the time of expected first detection of this antibody.

	samples with low Ct values were negative for virus culture.	
Owusu (2021) (38) Prospective cohort study USA Mar-May 2020	Collected serial nasopharyngeal specimens at various time points from individuals (n=109) with rRT-PCR-confirmed COVID-19. Viral culture (Vero CCL-81 cells) was attempted for rRT-PCR-positive nasopharyngeal specimens (n=35) collected ≥10 days after symptom onset. Participants were classified into three categories based on their viral RNA shedding duration: Persistent (≥14 days), not persistent (<14 days), or indeterminate.	- The Ct values of the 35 specimens collected ≥10 days after symptom onset ranged from 26.3-38.4. - Culture was not successful for any of these specimens.
Folgueira (2021) (36) Cross-sectional study Spain Feb 2021*	Respiratory samples (186 nasopharyngeal exudates and seven bronchial aspirates) were processed by rRT-PCR (targeting the E gene) and cell culture (Vero E6 cells) from asymptomatic (n=11), mild (n=91) and severe (n=87) patients, obtained at various times from clinical diagnosis to follow-up.	- In both the mild and severe groups, the samples that showed viral replication had significantly lower median Ct values than the samples without viable virus: 23.3 (IQR: 20.5–28.0) vs. 36.4 (IQR: 31.8–39.1), respectively, for mild COVID-19 and 27.7 (IQR: 23.2–30.0) vs. 33.0 (IQR: 30.4–38.0), respectively, for severe COVID-19) ($p < 0.001$). - The samples with Ct ≤ 25 in both patient groups showed viable virus at a rate >90%. However, even the samples with Ct ≥ 35 could harbour viable virus (5% for mild COVID-19 and 15% for severe illness).
Felix (2021) (26) *Preprint* Cross-sectional study Brazil Feb 2021*	Patients with confirmed mild COVID-19 were invited to participate by providing nasopharyngeal (NP) samples at the day 10 if illness (n=53). Cell-cultured SARS-COV-2 RT-PCR (targeting the S gene) positive respiratory samples (n=29) at 10 days post symptom onset in VeroE6 cells. After two passages, cytopathic effect and cycle threshold lower than that obtained in the original sample were used to determine positivity of culture.	- Forty patients (79%) were SARS-CoV-2 positive by RT-PCR at day 10 (79%) with the median Ct of 25.7 (range 12-32). Of these, 29 were submitted for culture testing. - Culture was successful for 24% (7/29) of the samples tested. - The positivity in cell culture was strongly associated with low Ct values in clinical samples. Mean Ct value was 20 (IQR 16.5-21) in culture positive samples vs. 29 (IQR, 24-32.3) in culture negative samples, $p<0.0001$. - Two patients for which culture was successful reported having no symptoms on day 10.

Marot (2021) (27) LTE Cross-sectional study France Aug-Sept 2020	Conducted real-time RT-PCR assays to detect the presence of the viral E (envelope) subgenomic RNA and E negative-strand RNA in clinical samples. Also attempted to isolate virus via culture (Vero CCL-81 cells, incubated 2-7 days) from samples to associate the presence of positive-stranded replicative intermediate RNAs (RIs) with the detection of viable virus. Data were obtained from 61 immunocompetent healthcare workers (HCWs) diagnosed with SARS-CoV-2 infection by RT-PCR on nasopharyngeal samples.	- No isolate was recovered via culture when Ct > 28 (i.e., viral load below 5.83 log_{10} copies/mL).
Piralla (2020) (28) Cross-sectional study Italy Apr-Aug 2020	A series of nasal swabs collected from convalescent patients positive for SARS-CoV-2 RNA detected by rRT-PCR (targeting the E or N gene) with Ct >30 were included in the study (n=387). Cell culture (Vero E6 cells incubated up to 7 days) was conducted to investigate the infectious potential of samples. Note: No time point (i.e., day of illness) was provided for when samples were collected, however it is specified that all samples were drawn from clinically recovered patients.	- The median Ct value of convalescent samples was 36.8 (range: 30.0–39.4). For the E gene, the median Ct value was 36.9 (range 30.0–39.4) while the N gene was 35.5 (range 32.0–39.4). - Culture of convalescent specimens was successful for only 9 samples (2.3%, 9/387). - The median Ct value of culture-positive samples was not significantly different from that observed in culture-negative samples (35.6 vs. 36.9, p = 0.37).
Romero-Gómez (2020) (29) LTE	Investigated samples obtained from patients with SARS-CoV-2, comparing the results obtained by RT-PCR with the growth capacity of the virus via cell culture (Vero E6 cells, incubated for 4 days). 72	- Culturable samples had significantly lower Ct values (<30) than those with non-culturable samples (>30) (p<0.0001, see figures 1&2 in article). - Isolation of virus in culture was successful in samples with Ct between 21.54 and 37.73. - The highest Ct values in samples with positive cultures were found to be 36.08, 37.73 and 37.41 for

Cross-sectional study Spain Feb-June 2020	nasopharyngeal specimens taken at various time points of infection from 66 patients were analysed in this study. 17 samples were successfully isolated.	the ORF1ab, N and S genes, respectively, taken 1 day post symptom onset from a patient with a cough and fever.
Jaafar (2020) (30) LTE Cross-sectional study France* May 2020	Performed 250,566 SARS-CoV-2 RT-PCR tests on nasopharyngeal samples (Ct values based on the E gene). 13,161 were positive and 1,941 isolates were obtained via culture (Vero E6 cells).	- At Ct = 25, up to 70% of patients remain positive in culture. At Ct = 30 this value drops to 20%. At Ct = 35, the value used to report a positive result for PCR, <3% of cultures are positive.
Kim (2021) (31) LTE Case series South Korea Feb-Jun 2020	Clinical and virological characterization of 21 hospitalized patients. Viral RNA was quantitated using rtRT-PCR (targeting the N gene) and viral cultures were conducted via plaque assay (vero cells) until at least two consecutive cultures showed no growth.	- Viable SARS-CoV-2 was cultured in 29/89 samples. - Viral culture was positive only in samples with a CT ≤ 28.4. - Median time from symptom onset to viral clearance in culture was 7 days. - The incidence of culture positivity decreased with an increasing time from symptom onset and with an increasing cycle-threshold value.
Vetter (2020) (35) Case series Switzerland Feb 2020	Clinical, virological, and immunological characterization of the first five patients assessed at the Geneva University Hospital (HUG), from the day of diagnosis until convalescence. SARS-CoV-2 was detected by rtRT-PCR in both the oropharyngeal swabs (OPS) and the nasopharyngeal swabs (NPS) of each patient. Viral culture was conducted using Vero E6 cells.	- Isolation of virus in culture was successful from both NPS and OPS during the first week of illness for four mild cases. - The mean viral load in samples affording successful isolation was $1.2x10^9$ copies/ml. SARS-CoV-2 could not be isolated in clinical specimens containing less than $1.4x10^6$ viral RNA copies/ml.
Lewis (2020) (39)	Investigated household transmission in 5 households with daily specimen collection.	- In multiple patients, low Ct values (<20) on days 2-4 post symptom onset coincided with onset of additional symptoms (chest pain, myalgia, and loss

Contact tracing study USA Apr 2020	During days 1–4, if a household contact had an inconclusive result (1 of 2 target gene regions positive for SARS-CoV-2 by rRT-PCR) or positive result (both target gene regions positive) the associated specimen and all subsequent daily specimens from the person were submitted for viral culture to evaluate infectiousness. Specimens positive by rRT-PCR that were collected on day 14 with Ct values<35 were also cultured. Gene targets for rRT-PCR and methods for culture were not described.	of taste and smell) and positive viral cultures on both days. - Culture was not successful among specimens collected 14 days post symptom onset, despite positive rRT-PCR and Ct values <35.
Subgenomic RNA Studies (n=3)		
Rodríguez-Grande (2021) (32) Case series Spain Jan 2021*	Assessed samples of persistent RT-PCR positive cases (n=60) with >21 days since the first diagnostic RT-PCR for evidence of replicative virus as determined by subgenomic E gene RNA (SG RNA).	- SG RNA was detected in 12/60 cases (20%). Collection dates ranged from 28-79 days after onset in these samples. - In all cases with detectable SG RNA, Ct values for genomic RNA were <30, consistent with the values expected for an active virus. - The age range of subjects with prolonged viral shedding and SG viral RNA was quite wide and equally distributed between males and females. Seven were immunosuppressed. The severities of the COVID-19 episodes were mild (40%), intermediate (20%), and severe (40%). One case with SG RNA at day 25 was asymptomatic.
Marot (2021) (27) LTE Cross-sectional study France Aug-Sept 2020	Conducted real-time RT-PCR assays to detect the presence of the viral E (envelope) subgenomic RNA and E negative-strand RNA in clinical samples. Also attempted to isolate virus via culture (Vero CCL-81 cells, incubated 2-7 days) from samples to associate the presence of positive-stranded replicative intermediate RNAs (RIs) with	- No RIs were detectable in samples when the Ct > 33 (viral load below 4.34 $\log_{10}$ copies/mL) - The ratios of mean normalized RIs per genome indicate a high level of viral replication during the first 5 days from symptom onset, followed by a significant decline.

	the detection of viable virus. Data were obtained from 61 immunocompetent healthcare workers (HCWs) diagnosed with SARS-CoV-2 infection by RT-PCR on nasopharyngeal samples.	
Hogan (2021) (33) Prospective and retrospective cohort studies USA Mar-Apr 2020 & July-Sep 2020	Developed a novel 2-step rRT-PCR specific to the minus strand of the envelope gene (actively replicating virus produces minus-strand RNA intermediates thus can be used as a proxy for potential infectiousness). Retrospectively collected a convenience set of longitudinal upper respiratory specimens with a broad range of Ct values. For the prospective phase of the study, they collected upper respiratory samples from 53 consecutive patients with confirmed SARS-CoV-2 infection. Samples were collected a median of 9 days (IQR: 4–18 days) after symptom onset.	- Minus-strand RNA was detected in 41 (28.1%) patients. - The median Ct value was significantly lower in samples with detected minus-strand RNA (20.7) than those in which the minus strand was not detected (33.2, $p<0.01$). - Minus-strand RNA was detected in two immunocompentent inpatients >10 days post symptom onset with high Ct values (~39). Minus-strand SARS-CoV-2 RNA was detected up to 30 days after symptom onset in an immunocompromised patient.

LTE= letter to editor

ASSOCIATION BETWEEN VIRAL LOAD AND TRANSMISSION RISK

- Viral loads appear to peak on average 5 days post expsoure and 1-2 days post symptom onset, with a short period (<2 days) of high transmission risk and transmission potential being greatly diminished by 7-10 days post symptom onset (40-42). During peak viral load, highly infectious cases can shed tens to thousands of SARS-CoV-2 virions/min (24).

- Several observational studies were conducted that investigated household/cluster transmission. In line with the culture findings from the section above, cases with higher viral loads (i.e., lower Ct values) were more likely to transmit SARS-CoV-2.
    - Index cases with high viral load (>10^6 copies/uL) were more likely to transmit SARS-CoV-2 to close contacts (OR 4.9, 95% CI: 1.3-18, p=0.02) (43). The secondary attack rate was reported to range from 12% when the index case had a viral load ≤$1x10^6$ copies/mL to 24% when the index

case had a viral load of ≥1x10^{10} copies/mL (adjusted odds ratio per $\log_{10}$ increase in viral load=1.3, 95% CI: 1.1-1.5) (44).

- Regression models demonstrated that cases resulting in secondary transmission had higher median viral loads than that of cases that did not transmit SARS-CoV-2 (45). Two retrospective cohort studies conducted in US university residences also found that index cases resulting in secondary transmission had higher viral loads (up to 6.5 fold) than cases that did not cause secondary transmission (46, 47). Although both symptomatic and asymptomatic cases exhibited average Ct peaks around Ct 19-22, the Ct values were significantly lower in symptomatic cases (range: 12-36 vs. 14-37), indicative of reduced viral load among asymptomatic cases (47).
- A cross-sectional study comprising the full population of Denmark found that index cases with a Ct <20 had a transmission risk 1.89 times higher than an index case with a Ct >25 (48). The index case had a Ct value of >30 in 39% of secondary cases, but transmission risk was significantly higher if the Ct values were <28. Index cases with high Ct values (>35) did occur, but the transmission risk was much lower (8%).
- The majority of secondary household cases were detected within 10 days post symptom onset of the index case, aligning with the timing of recovery of replicative virus findings noted in section above (43).
- One study found that clusters with high viral loads (>10^6 copies/mL) were considerably larger than clusters with subjects showing a lower viral load (17 infected individuals vs 3 within cluster, p<0.001) (49).

- There were few studies that reported on how the relationship between viral load and transmission risk varied by presence of symptoms, severity of symptoms, or risk factors (e.g., age, chronic health conditions).
  - A systematic review found that adult, pediatric, symptomatic/presymptomatic and asymptomatic COVID-19 cases show similar respiratory viral load distributions during the infectious period (24). Interestingly, a larger population study found that older age was positively associated with transmission risk, even after controlling for viral load (i.e., age was a better predictor of transmission risk than viral load) (48).
  - High viral loads (Ct<20) prior to symptom onset among cases that transmitted SARS-CoV-2 presymptomatically have been reported (50). The longer the incubation period, the larger the fraction of presymptomatic viral load, and thus a higher likelihood of presymptomatic transmission (41).
  - High viral loads have been reported both in the pre-symptomatic period and up to 8-10 post-symptom onset. It is during this time when Ct values are low (<30) that there is a high probability of transmission.

Table 2: Studies that investigate association between viral load and transmission risk (n=12).

STUDY	METHOD	KEY OUTCOMES
Chen (2020) (24) *Preprint* Systematic review and modelling study Canada* Dec 2020*	A systematic review was conducted to capture studies published up to August 7, 2020 (n=64) in order to develop a comprehensive dataset of respiratory viral loads (rVLs) of SARS-CoV-2. A meta-analysis and model was then conducted to investigate individual infectiousness by shedding viable virus via respiratory droplets and aerosols. SARS-CoV-2 rVLs was analyzed across age and symptomatology subgroups as well as disease course.	- At the 90th case percentile (cp) throughout the infectious period, the estimated rVL was 8.91 (95% CI: 8.83-9.00) log10 copies/ml. - Age and symptomatology minimally influenced case variation in SARS-CoV-2 rVL during the infectious period. Adult, pediatric, symptomatic/presymptomatic and asymptomatic COVID-19 cases showed similar rVL distributions, with standard deviations of 2.03, 2.06, 2.00 and 2.01 log10 copies/ml, respectively. - The mechanistic model showed that SARS-CoV-2 rVL increased exponentially after infection, peaked around 1 day post symptom onset and then diminished exponentially. Highly infectious cases can shed tens to thousands of SARS-CoV-2 virions/min, especially between 1-5 days post symptom onset.
Cerami 2021 (43) *Preprint* Prospective cohort study USA Apr-Oct 2020	Enrolled COVID-positive persons (n=102) and their household members (n=213) to study SARS-CoV-2 transmission within households. Households were enrolled a median of 6 days from onset of symptoms in the index case. Secondary cases were detected either by RT-qPCR (targeting N1, N2, and RNase P) of a nasopharyngeal swab on study day 1 and weekly nasal swabs (days 7, 14, 21), or based on seroconversion by day 28.	- The majority of secondary household cases were detected within 10 days post symptom onset of the index case. - Index cases with high viral load (>10^6 viral copies/ul) at enrollment were more likely to transmit virus to household contacts during the study (OR 4.9, 95% CI 1.3-18, p=0.02). - Viral load was correlated within families, meaning persons in the same household were more likely to have similar viral loads, suggesting an inoculum effect. These differences were not attributable to the D614G mutation in the SARS-CoV-2 spike protein, as the vast majority of isolates genotyped contained this mutation (98%).
Bjorkman (2021) (46) Retrospective cohort study USA	Analyzed the transmission of COVID-19 in residence halls based on the weekly RT-qPCR (targeting the E gene) screening of residential	- The average viral load was 6.5-fold higher in rooms with likely transmission (mean Ct=26.2) than in rooms without transmission (mean Ct=28.9).

Aug-Nov 2020	students. Each Ct whole unit is a factor of 2 in RNA copies per ml. Investigated the extent to which the timing of cases supported inter-roommate transmission.	- These cases spanned a range of over 7 orders of magnitude in viral load, with the highest load found in the likely transmission group (Ct=15.4) and the lowest found in the unlikely transmission group (Ct=40.6). The only cases with Ct greater than 34 (22 cases) were found in the unlikely transmission group.
Tian (2021) (47) *Preprint* Retrospective cohort study USA Sept-Oct 2020	Performed a total of 61,982 tests of 7,440 undergraduate students to determine whether the Ct value could differentiate the spreader from the non-spreader. Students were tested multiple times via RT-PCR (targeting the N, S and ORF1ab genes) over the study period and 602 cases were identified.	- 48.2% (94/195) of index cases had at least one contact who became SARS-CoV-2-positive, whereas 51.8% of the index cases (n=101) did not spread SARS-CoV-2 to their contacts. - Mean Ct values of the spreader and the non-spreader were nearly identical (peak at Ct = 18-21) but their median Ct values differed by almost one cycle, suggesting that spreaders had a lower Ct value than the non-spreaders (see Figure 1B&C in article). Ct range was slightly broader for the spreader (12-36) than that for the non-spreader (14-36). - Although both groups exhibited Ct peaks around Ct 19-22, the Ct values were significantly lower in symptomatic than those in asymptomatic cases (range: 12-36 vs. 14-37), indicative of reduced viral load among asymptomatic cases.
Shrestha (2021) (42) Retrospective cohort study USA Mar-Apr 2020	Evaluated transmission potential by examining viral load with respect to time since onset of symptoms in healthcare professionals infected with SARS-CoV-2 (n=230). Nasopharyngeal swabs were tested by RT-PCR (targeting the N1, N2, and N3 genes). The mean of the 3 Ct values from the three gene targets was considered the Ct for the test. Viral loads since symptom onset were predicted using the	- SARS-CoV-2 viral RNA load is very high within 2–3 days post onset of symptoms and falls rapidly by orders of magnitude within a few days (see Figure 1 in article). - Transmission potential of COVID-19 is greatly diminished by 7–10 days post onset of symptoms (see Figure 3 in article). Of the AUC spanning the interval from onset of symptoms to 30 days, 86.3% lie within the first 5 days, 96.9% within the first 7 days, and 99.7% within the first 10 days.

	regression model, with the area under the curve (AUC) representing the distribution of transmission potential over time.	
Marks (2020) (44) Retrospective cohort study Spain Mar-Apr 2020	This study was a post-hoc analysis of data collected in a cluster randomised trial that included individuals with qPCR confirmed COVID-19 and their close contacts. Factors associated with transmission were assessed by linear regression using all clusters of an index case for which quantitative viral load from nasopharyngeal swabs was available.	- The overall secondary attack rate was 17% (125/753 contacts) with a range of 12% when the index case had a viral load $\leq 1x10^6$ copies/mL to 24% when the index case had a viral load of $\geq 1x10^{10}$ copies/mL. - According to multivariate analysis, the odds of transmission were higher when the index case had a high viral load (adjusted odds ratio per $\log_{10}$ increase in viral load=1.3 (95%CI: 1.1-1.5). - 90% of transmission events occurred when the index case had a high viral load (≥5.1 $\log_{10}$ copies/mL). 50% occurred in clusters where the index case had a viral load of ≥8.8 $\log_{10}$ copies/mL. - Other factors associated with an increased risk of transmission were household contact and age of the contact. No association was observed with mask usage, age or sex of index case, or with presence of respiratory symptoms in index case.
Kawasuji (2020) (45) Case-control study Japan Apr-May 2020	COVID-19 patients who transmitted the disease to at least one other patient were analysed as "cases" (index patients, n=14) and compared with patients who were not the cause of secondary transmission (non-index patients, n=14, analysed as "controls"). Cases were confirmed and viral load quantified via RT-qPCR (targeting the N2 gene). The nasopharyngeal viral load time courses were assessed between the index and non-index symptomatic patients using non-linear	- Viral loads peaked soon after symptom onset, and then gradually decreased. Median time to viral clearance did not significantly differ between index and non-index patients: 21 days (IQR: 15–31) vs. 17 days (9-26), p=0.34. - The viral load at the time of initial sample collection was significantly higher in symptomatic vs. asymptomatic patients and in adult vs. children. - Regression models of symptomatic cases only (n=18) demonstrated that the median viral load of the index patients at onset was higher than that of the non-index patients: 6.6 log copies/µL (95%CI: 5.2-8.2) vs. 3.1 (1.5-4.8). This trend continued until 10 days post onset. When asymptomatic cases were also included in this model (n=10), the

	regression employing a standard one-phase decay model.	overall trend stayed the same: 3.3 log copies/µL (95% CI: 1.6-5.2) vs. 1.8 (-0.4-4.6), p=0.015.
Lyngse (2021) (48) *Preprint* Cross-sectional study Denmark Aug 25-Feb 10, 2021	Used comprehensive administrative register data from Denmark, comprising the full population and all SARS-CoV-2 tests, to estimate household transmission risk. RT-PCR (targeting the E-gene) was used and a test for SARS-CoV-2 was defined as positive if the Ct value was ≤38. 66,602 primary cases were identified, from which 99.6% had available Ct values. 103,389 secondary cases were identified.	- 25% of primary cases had a Ct value ≤25, 50% had a Ct value ≤28, and 75% had a Ct value ≤32. Ct value was similar across age groups. - There was an approximately linear decreasing relationship between Ct values and transmission risk. - The index case had a Ct value of >30 in 39% of secondary cases (but this is day of testing vs. an unknown peak viral load point- they did not have data to adjust for this). Primary cases with a Ct value of 38 had a transmission risk of 8% within the household. - Transmission risk was significantly higher if Ct values were <28. - A primary case with a Ct value of 18-20 has a transmission risk 1.89 higher than a primary case with a Ct value of 36-38. - Transmission risk had a negative association with age in children <20 and a positive association with age for those >20 yrs.
Ladoy (2021) (49) *Preprint* Surveillance study Switzerland Jan-Jun 2020	Characterized the dynamics of the first wave of SARS-CoV-2 infection in the canton of Vaud (western Switzerland) through the detection and the location of clusters using the results of SARS-CoV-2 RT-PCR tests (n= 33,651 tested, n=3,317 positive). A spatial scan approach was used to assess the importance of viral load in the evolution of the clusters and a Modified Space-Time DBSCAN algorithm was used to	- 1,684 space-time clusters were identified. - The majority of clusters had at least one person with a high viral load (>1 billion copies/ml). Clusters with such high viral loads were considerably larger (median of 17 infected individuals) than clusters with subjects showing a viral load lower >1 million copies/ml (median of 3 infected individuals, p<0.001). - Clusters involving younger individuals had the highest viral loads, while clusters composed of older individuals had low to medium viral loads. - There were 20 clusters in which the viral load of the three first cases were all below 100,000 copies/ml, suggesting that in some

	characterize the diffusion dynamics of transmission clusters.	instances, even subjects with less than 100,000 copies/ml may still be contagious.
Park (2020) (50) Case series South Korea -> Israel Feb 2020	An outbreak of SARS-CoV-2 among 39 pilgrim travelers was investigated. Ten confirmed cases without symptoms at the first sampling dates (2 post-symptomatic, 4 pre-symptomatic, and 4 asymptomatic) were selected for follow-up respiratory tract sample RT-PCR tests (targeting the E gene).	- Available Ct values from specimens of the lower respiratory tract were significantly lower in cases without symptoms (median, 22.8; IQR, 20.1–25.4) than in cases with symptoms at the first sampling dates (median, 27.9; IQR, 23.4–30.4). The viral loads gradually decreased over time and were not different between symptomatic and asymptomatic cases. - The highest viral load (Ct<20) was observed from the first sample of a case collected 7 days before symptom onset. Transmission occurred from this case to a close contact during the presymptomatic period.
Goyal 2021 (40) Modelling study USA* Feb 2021*	Developed a transmission simulation framework to analyze the contribution of viral load to observed epidemiologic transmission metrics SARS-CoV-2. This process included within-host modeling of viral loads, simulations of exposures and possible transmissions based on various transmission dose response curves, testing of various parameter sets against epidemiologic data and exploratory analyses with the best fitting model.	- Simulations demonstrated an upper airway viral load $<10^4$ SARS-CoV-2 RNA copies is very unlikely (~0.00005%) to lead to transmission. Transmission is much more likely (39%) given an exposure to an infected person who is shedding $>10^7$ RNA copies, and 75% given an exposure to an infected person with a viral load of $>10^8$ RNA copies. - There is an inflection point between 10^6 and 10^7 RNA copies, after which multiple transmission events becomes much more likely from a single person (i.e., super spreading event). - Infected persons are likely to be most infectious (viral load above TD50) for a 0.5–1.0 day period between days 2 and 6 post infection. This variability is likely attributable to heterogeneity in incubation period rather than timing of peak viral load.
Ke (2020) (41) *Preprint* Modelling study USA*	Developed data-driven within-host models of SARS-CoV-2 infection. Analyzed the relationship between viral load in the lower and upper respiratory trans (LRT & URT) and potential	- Respiratory viral load peak on average 5.2 days (SD: ±1.3 days) and 5.4 days (±1 day) post infection in the URT and the LRT, respectively, and on average 2 days (±0.2 day) and 2.1 days (±1.2 days) post symptom onset.

Sep 2020	infectiousness via a probabilistic model using existing epidemiological evidence (from 8 cases). Develop several more models to explain the prolonged period of virus infection in the LRT.	- The longer the incubation period, the larger the fraction of presymptomatic viral load (See Fig. 2; $p<0.001$), and thus leads to a higher fraction of presymptomatic transmission. - Model demonstrates that the logarithm of viral load (rather than absolute viral load) is an appropriate surrogate for infectiousness (i.e., viral growth is a better predictor of presymptomatic transmission than viral load measurement alone). - Spatial dissemination in the lungs is an important process in sustaining prolonged high viral loads in the LRT.

Methods:

A daily scan of the literature (published and pre-published) is conducted by the Emerging Sciences Group, PHAC. The scan has compiled COVID-19 literature since the beginning of the outbreak and is updated daily. Searches to retrieve relevant COVID-19 literature are conducted in Pubmed, Scopus, BioRxiv, MedRxiv, ArXiv, SSRN, Research Square and cross-referenced with the COVID-19 information centers run by Lancet, BMJ, Elsevier, Nature and Wiley. The daily summary and full scan results are maintained in a refworks database and an excel list that can be searched. Targeted keyword searching is conducted within these databases to identify relevant citations on COVID-19 and SARS-COV-2. Search terms used included: Shedding, Viral dynamics, Viral RNA dynamics, Viral clearance, Viral RNA clearance, Viable, Culture, Infectivity, Infectious Period, Communicability period, Period of communicability, Viral load, Viral RNA load, Infectiousness. This review contains research published up to March 31, 2021. Each potentially relevant reference was examined to confirm it had relevant data and relevant data is extracted into the review.

Peer-review

This document underwent peer-review by a subject matter expert, and editorial and science to policy review by the Office of the Chief Science Officer.

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