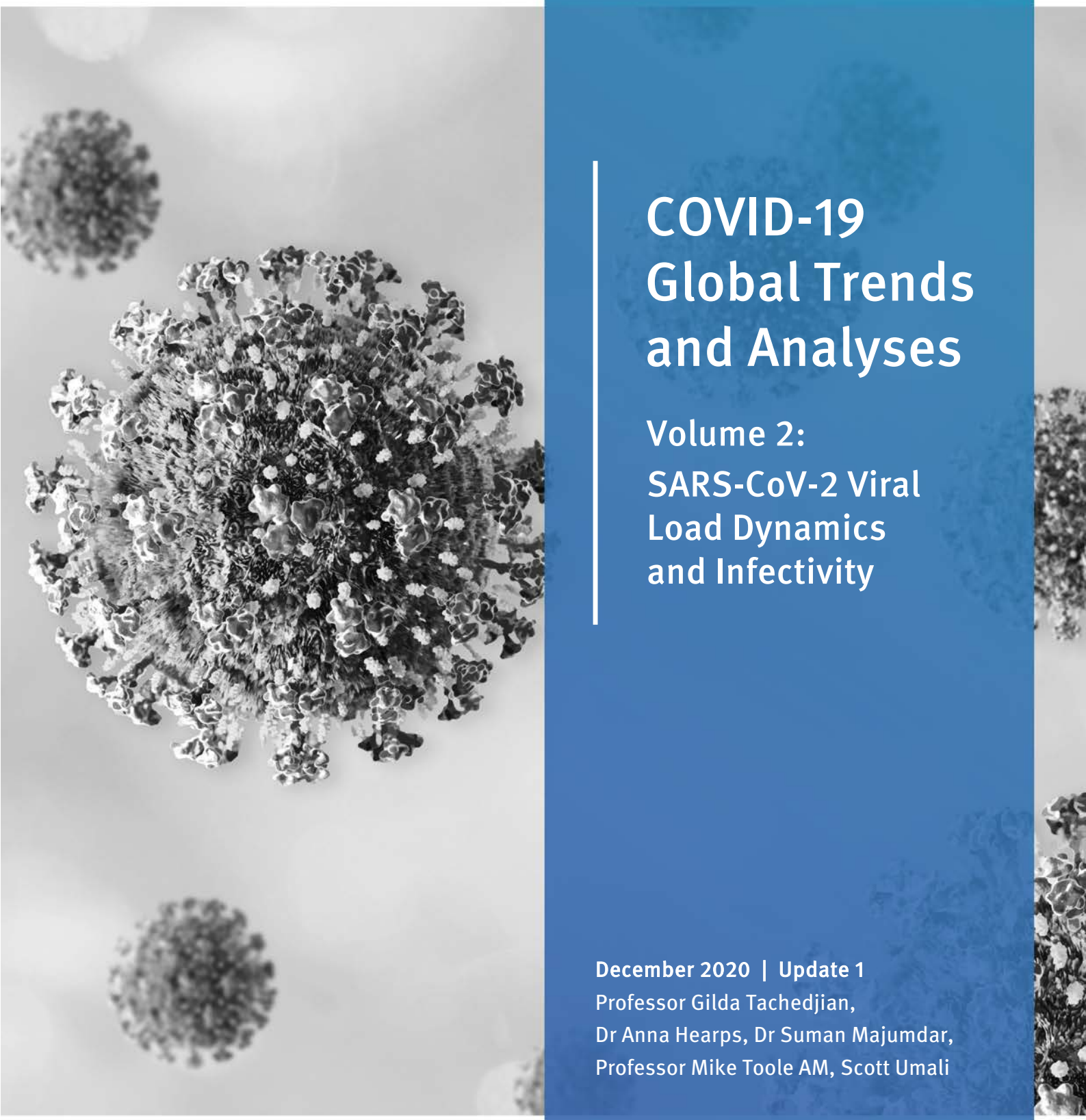

COVID-19 Global Trends and Analyses

Volume 2: SARS-CoV-2 Viral Load Dynamics and Infectivity

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SUMMARY

COVID-19 GLOBAL TRENDS AND ANALYSES | 11 Nov - 7 Dec 2020

- The **global** total number of reported cases has surpassed 67 million - an increase of 17 million in four weeks - and more than 1.5 million deaths as of 8 December. This exceeds the leading infectious killers in 2019 - 1.4 million deaths from tuberculosis, 690,000 from HIV/AIDS and 380,000 from malaria.
- In **Europe**, the number of new daily cases has been in decline for at least two weeks. Exceptions include Russia, Turkey, Poland, Ukraine, Romania, Croatia and Sweden.
- In the **United States,** the number of cases has surpassed 15 million (six days after reaching 14 million) and the death toll is more than 290,000. More than 2,800 deaths were reported on 3 December, an all-time high. Likewise, the more than 100,000 people currently hospitalised is the highest ever.
- **Indonesia** has reported more than 580,000 cases and 17,800 deaths. The country continues to report 5,000 to 6,000 new cases daily but reached a record peak of 8,369 on 3 December.
- **Japan, South Korea and Hong Kong** are each experiencing third waves of cases.
- **Vietnam** has reported three new community cases after more than 80 days of zero local transmission. The new cases are linked to an infected flight attendant.
- **Myanmar** continues to report around 1,500 new cases daily.
- **Papua New Guinea** has reported 65 new cases in the past 14 days, with a total of 671 cases.
- **Victoria** has reported zero new cases for 36 consecutive days. Restrictions continue to be gradually eased while masks remain mandatory to carry at all times and wear on public transport, taxis and rideshares, and retail.
- **New South Wales** has reported one new locally acquired case, a hotel quarantine worker, after zero new cases for 26 days.
- **South Australia** has reported no new local cases for seven days after a cluster of more than 30 cases following the infection of a hotel quarantine worker.

SCIENCE AND RESEARCH | SARS-CoV-2 VIRAL LOAD DYNAMICS AND INFECTIVITY

Summary

SARS-CoV-2 viral load dynamics, shedding, and infectiousness are critical factors for viral transmission and for implementing strategies to control the COVID-19 pandemic. However, our understanding of the dynamics and duration of viral shedding for SARS-CoV-2 is incomplete and ongoing.

A systematic review and meta-analysis published in November 2020 of 79 studies reports that viable SARS-CoV-2 is not detected beyond nine days of illness despite persistently high viral loads and evidence of prolonged shedding in respiratory and stool samples. SARS-CoV-2 viral load peaks at the time of symptom onset or during the prodromal stage of illness. While no study in this review confirmed that viral load peaked prior to the onset of symptoms, the patient may be infectious during the pre-symptomatic phase. These data are consistent with observations in contact tracing where highest transmission risk occurs very early in the disease course spanning a few days before and within 5 days of symptom onset.

There is considerable evidence that high viral loads are associated with severe COVID-19 illness. One study showed a much higher proportion of patients on a ventilator (44%) had detectable viremia compared with those on supplemental (19%) or no oxygen (0%).

Definitions

Viral load: Viral load refers to the amount of virus in an infected person's blood, plasma or other samples, such as from the respiratory tract. This is usually expressed as the number of viral particles in each millilitre of blood. Higher viral load can have different implications for different viruses but typically means the infection is progressing.

Viral shedding: Viral shedding refers to the expulsion and release of virus progeny following successful reproduction during a host-cell infection. Once replication has been completed and the host cell is exhausted of all resources in making viral progeny, the viruses may begin to leave the cell by several methods. The term is used to refer to shedding from a single cell, shedding from one part of the body into another part of the body, and shedding from bodies into the environment where the viruses may infect other bodies.

RT-qPCR: A reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) test is used to determine viral load in samples. The method's name derives from the fact that the amplification of DNA by polymerase chain reaction (PCR) is monitored in real-time. It is a quantitative method in contrast to conventional PCR, meaning that it enables the determination of exact amounts (relative or absolute) of amplified DNA in samples.

Viral culture: Viral culture is a laboratory technique in which samples of a virus are placed into different cell lines which the virus being tested for is able to infect. If the cells show changes, known as cytopathic effects, then the culture is positive.

Viral load over time

Viral load and its temporal profile are useful predictors for disease severity and progression, used in the context of other diseases such as HIV, Ebola, influenza and other non-respiratory viral infections[1]. In the upper respiratory tract, the **viral load of SARS-CoV-2 peaks in the first week of illness** in contrast to SARS-CoV and MERS-CoV where the viral load peaks at days 10-14 and 7-10, respectively[1]. The early peak of SARS-CoV-2 at the time of symptom onset to 5 days of illness likely explains the ability of this virus to spread more efficiently compared to SARS-CoV and MERS-CoV. Asymptomatic (including pre-symptomatic) and symptomatic individuals appear to have similar viral loads at the start of SARS-CoV-2 infection, although asymptomatic individuals demonstrate faster viral clearance[1], which is similar to what is observed for influenza and MERS-CoV[2,3].

While the **reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR)** test is used to determine viral load in samples, detection of viral genomic RNA cannot be used to infer virus infectiousness, especially following resolution of symptoms. A positive association was observed for SARS-CoV-2 shedding duration and age, with men exhibiting a longer duration of shedding. To help limit transmission in the community and in healthcare settings, isolation should be initiated at the start of symptoms, which can be mild and atypical, and precede the characteristic SARS-CoV-2 symptoms of a fever and cough. In addition, testing of asymptomatic contacts in high-risk workplaces, such as healthcare settings and meatpacking sites, is a warranted precaution.

Novel techniques for the specific detection of active replication are currently being evaluated[4,5]. SARS-CoV-2 viral load in respiratory samples and particularly in blood is associated with disease severity and mortality, and therefore may represent a useful predictive biomarker for identifying individuals at risk of severe clinical disease.

Measuring Viral Load

SARS-CoV-2 diagnosis, screening and surveillance rely on measuring viral RNA. The quality of sampling is an important determinant of accurate detection. Nasal, nasopharangeal and oropharangeal swabs have had different detection rates[6]. Viral load dynamics and shedding are critical factors for viral transmission as well as predicting disease severity[7]. Viral load is determined by employing the RT-qPCR assay to detect the presence of the SARS-CoV-2 RNA genome, typically targeting the N or E gene of SARS-CoV-2[8]. This assay indicates at what number of cycles of amplification of PCR or 'cycle threshold' (Ct) the sample is detected, with **a lower Ct indicating a higher amount of viral RNA** in the sample. Viral load can be quantified as the actual viral RNA copies in a sample by running known viral RNA copy numbers in the assay to generate a standard curve. Studies to measure SARS-CoV-2 and its relationship with infectious virus have utilised either Ct or RNA copies[9].

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1 https://doi.org/10.1016/S2666-5247(20)30172-5 (Cevik M et al 2020 Lancet Microbe)

2 https:// doi: 10.3201/eid2207.160040 (Al Hosani et al 2016 Emerg Infect Dis 2016 22:1162)

3 https://doi: 10.1093/cid/ciw841 (Dennis KM Ip et al 2017 Clin Infect Dis 64:736)

4 https://doi.org/10.1101/2020.06.01.20119750. medRxiv preprint posted August 16, 2020. (Alexandersen S et al)

5 https://wwwnc.cdc.gov/eid/article/26/11/20-3219_article (Ranawaka APM et al Emerging Infectious Diseases

6 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7494432/

7 http://dx.doi.org/10.1136 bmj.m1443 (Zheng S et al 2020 BMJ 369: m1443)

8 https://doi.org/10.1016/j.jinf.2020.10.017 (Dahdouh E et al 2020 Journal of Infection)

9 https://doi.org/10.1007/s10096-020-03913-9 (La Scola et al 2020 Eur J Clin Micro and Infec Dis 39: 1059-1061)

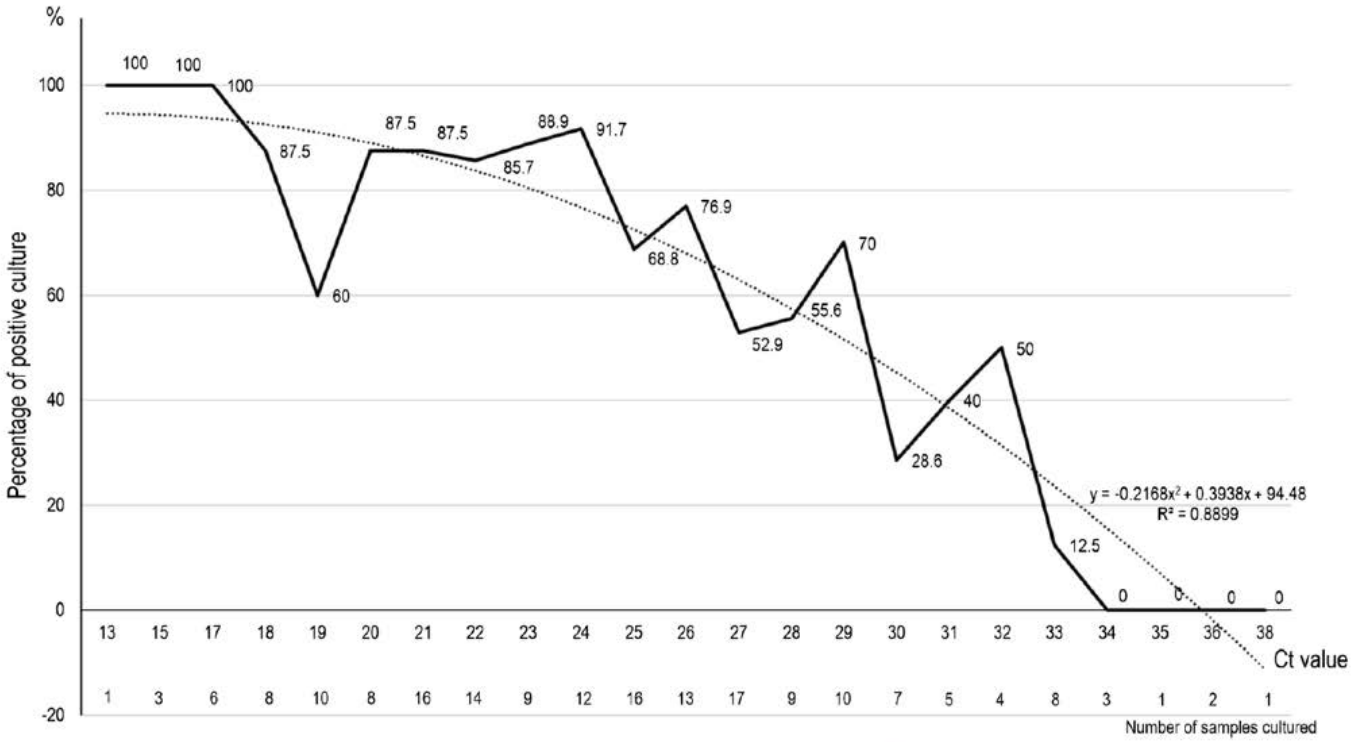

Figure 1: *Percentage of positive viral culture of SARS-CoV-2 PCR-positive nasopharyngeal samples from COVID-19 patients* (n=183)*, according to Ct value (plain line). The dashed curve indicates the polynomial regression curve8*

Relationship between viral load and infectivity

Detection of viral RNA does not necessarily mean that the virus is infectious. The gold standard for determining infectivity is to demonstrate that the virus can infect and reproduce in cell culture assays, with Vero E6 cells typically used for propagation of SARS-CoV-2[8]. Analysis of 183 nasopharyngeal samples from COVID-19 positive donors by qRT-PCR found 124 had infectious virus in cell culture and that there was a relationship between Ct value (i.e. viral load) and culture positivity (Fig 1)[8], with a decreasing ability of samples with lower viral loads to be cultured. Another study evaluating 690 respiratory samples from 129 patients hospitalised with COVID-19 identified that a **viral load greater than 10,000,000 SARS-CoV-2 RNA copies/ml was independently associated with being able to culture infectious virus** from respiratory tract samples [odds ratio [OR]; CI 14.7 (3.57-58.1; p<0.001)][10]. Other studies have indicated infectious virus can rarely be cultured from samples with viral loads less than approx. 1,000,000 RNA copies/ml[5, 9,11]. These data suggest that quantifying the viral load of genomic SARS-CoV-2 RNA has potential to infer the presence of infectious virus. It should be noted that these studies exclusively looked at symptomatic and usually hospitalised patients. No studies have shown positive viral culture or transmission beyond nine days after the onset of symptoms.

10 https://doi.org/10.1101/2020.06.08.20125310 medRXivs preprint posted June 9, 2020 (van Kampen et al)

11 https://doi.org/10.1038/s41586-020-2196-x (Wolfel R et al Nature 581)

Subgenomic RNA and viral infectivity

Several studies have used RT-qPCR to infer the presence of actively replicating virus by detecting SARS-CoV-2 subgenomic RNA in respiratory samples[5,9,10]. The premise for the assay is that subgenomic RNA is only present when the virus is actively replicating in the cell[4]. However, subgenomic RNA, as well as SARS-CoV-2 genomic RNA, are protected within membranes inside the cell and may be present well after active viral replication has ceased. Indeed, **the correlation between infectious virus and presence of subgenomic RNA is mixed**. In a study of severely ill COVID-19 patients by van Kampen et al, detection of subgenomic RNA was a poor predictor of cultures that were positive for infectious virus (positive predictive value of 37.5 per cent)[9]. Accordingly, subgenomic RNA may not a suitable marker for inferring active virus replication particularly if sampling cellular material[4] however further studies using longitudinal samples will be needed to resolve this issue.

SARS-CoV-2 viral load dynamics, duration of viral shedding, and infectiousness

A systematic review and meta-analysis of 79 studies (5,340 individuals) published between Jan 1, 2003 and June 6, 2020 has provided a comprehensive analysis of viral dynamics of SARS-CoV-2 viral shedding and the presence of viable virus. The study also included a comparison with SARS-CoV-1 viral load from eight studies (1,858 individuals) and MERS-CoV viral load from 11 studies (799 individuals)[1]. Viral RNA was quantified in the upper respiratory tract, lower respiratory tract, stool and in serum samples. The **peak SARS-CoV-2 viral load in the upper respiratory tract occurred in the first week of illness** and later in the lower respiratory tract. This observation is distinct from SARS-CoV and MERS-CoV where virus in peaks at days 10-14 and 7-10, respectively.

The ability to detect viral load peak as well as infectious SARS-CoV-2 virus within the first week of illness at the time of symptom onset or during the prodromal stage of illness indicates that **infected individuals are likely to be the most infectious during this period**[13]. These data are consistent with observations in contact tracing where highest transmission risk occurs very early in the disease course spanning a few days before and within 5 days of symptom onset and underscores the importance of **immediate isolation upon symptom onset to prevent transmission**[12]. However, in contrast to modelling studies that have estimated that there is potentially a peak SARS-CoV-2 viral load before the onset of symptoms, **none of the studies identified a pre-symptomatic viral load peak**[2]. In several studies, similar viral loads were observed at the start of infection in asymptomatic and symptomatic patients infected with SARS-CoV-2; however there appears to be **faster clearance of virus in asymptomatic individuals**. Children infected with SARS-CoV-2 generally experience a milder clinical disease and are not thought to be major drivers of transmission[13]. There are limited data on SARS-CoV-2 viral load in children, but a recent family case study suggests children may clear SARS-CoV-2 without ever exhibiting detectable virus[14].

12 https://jamanetwork.com/journals/jamainternalmedicine/fullarticle/2765641

13 https://doi: 10.3205/dgkh000359 (Heudorf et al 2020 GMS Hyg Infect Control)

14 https://doi: 10.1038/s41467-020-19545-8 (Tosif et al 2020 Nat Commun)

Duration of viral shedding

The mean duration of SARS-CoV-2 RNA shedding in the upper respiratory tract was 17.0 days (95% CI 15.5-18.6; 43 studies, 3,229 individuals) and in the lower respiratory tract 14.6 days (9.3-20.0; seven studies, 260 individuals). In the stool the mean duration of SARS-CoV-2 RNA shedding was 17.2 days (14.4 - 20.1; 13 studies, 586 individuals) and 16.6 days (3.6 - 29.7; two studies, 108 individuals) in serum samples. The **maximum duration of SARS-CoV-2 RNA shedding** was 83 days in the upper respiratory tract, 59 days in the lower respiratory tract, 126 days in stools and 60 days in serum. Pooled mean SARS-CoV-2 shedding duration was positively associated with age (slope 0.304 [95% CI 0.115 - 0.496]; p=0.0016) and male sex.

Despite some patients with SARS-CoV-2 infection experiencing prolonged shedding in the upper respiratory tract of up to 83 days, **no study detected live virus beyond day 9 of illness**, even in the presence of persistently high viral loads[1]. Therefore, the presence of virus in samples does not correlate with infectivity. In most cases, guidelines no longer require repeat testing to determine if an individual is no longer infectious provided there has been clinical resolution or improvement[15]. Isolation timelines reflecting viral dynamics could be counted from symptom onset for 10 days in patients with non-severe disease.

SARS-CoV-2 RNA can be detected in stool samples for extended periods with high viral loads even after three weeks of illness[1]. To date, only two studies have been able to show infectious virus in stool samples[16,17]. Accordingly, the role of faecal SARS-CoV-2 RNA in viral transmission is unknown but is not thought to be a major contributor to viral transmission. Whilst it cannot differentiate between active viral transmission and shedding from non-*infectious individuals,* ***the detection of viral RNA in waste water is a sensitive*** and useful tool for monitoring the presence of SARS-CoV-2 within the population and monitoring whether transmission is increasing or decreasing. Monitoring SARS-CoV-2 RNA in wastewater has been shown to track with infection dynamics and may even provide an early indicator of rising community transmission[18] and has been used in other infectious diseases surveillance and control such as polio.

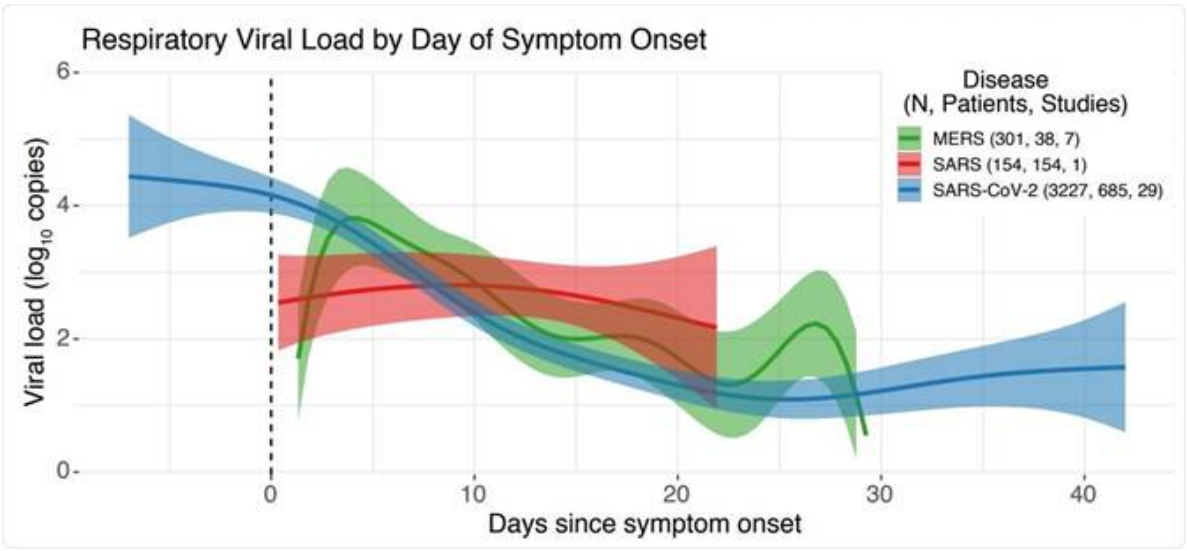

Figure 2: SARS-CoV-2, MERS-CoV, and SARS-CoV had median viral shedding durations of 4.8, 4.2, and 1.2 days after symptom onset (pre-publication data)[19].

15 https://www.who.int/news-room/commentaries/detail/criteria-for-releasing-covid-19-patients-from-isolation

16 https//doi: 10.3201/eid2608.200681 (Xioa F et al 2020 Emerg Infect Dis 26:1920)

17 https//doi: 10.1001/jama.2020.3786 (Wang W et al 2020 JAMA)

18 https://doi.org/10.1038/s41587-020-0684-z (Peccia et al 2020 Nat Biotech).

19 https://www.medrxiv.org/content/10.1101/2020.09.28.20202028v1

Association of SARS-CoV-2 viral load with disease severity

A number of studies have reported higher SARS-CoV-2 viral loads and/or longer duration of viral shedding in respiratory samples from individuals with more severe forms of COVID-19 disease (Figure 2)[1,6,20]. The presence of detectable viral SARS-CoV-2 RNA in the blood (viremia) is also more common in severe disease, and may be indicative of disease severity[17,21]. In a study of 88 individuals hospitalised with SARS-CoV-2, 44 per cent of those on a ventilator had detectable SARS-CoV-2 viremia as compared to 19 per cent requiring supplemental oxygen only and 0 per cent requiring no oxygen[17]. **Blood viral loads are positively associated with systemic hyper-inflammation (a key feature of severe COVID-19 disease)** and lower total lymphocyte counts[17,18], whilst detectable plasma viremia at the time of hospitalisation was shown in one study to be associated with a significantly elevated risk of death (odds ratio 5.5, p=0.02)[17]. Individuals with detectable viremia also have a higher prevalence of myocardial injury than aviremic individuals[22]. These data suggest SARS-CoV-2 **viral load in blood may be a clinically useful prognostic marker** to stratify individuals at high risk of adverse disease outcomes.

20 doi: 10.1038/s41467-020-19057-5 (Fajnzylber et al 2020 Nat Commun)

21 doi: 10.1093/cid/ciaa449 (Chen et al 2020 Clin Infect Dis)

22 https://doi.org/10.1016/j.amjmed.2020.09.046 (Siddiqi et al Am J Med)

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