Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Viable virus shedding during SARS-CoV-2 reinfection

The reinfection risk in individuals previously infected with SARS-CoV-2 is about a fifth of those never infected.[1,2] Whether reinfected individuals shed viable virus has been identified as an important question relevant to pandemic control.[3]

In a prospective cohort study in *The Lancet Respiratory Medicine*,[2] we identified 19 cases of reinfection in people who at study entry were seropositive for both SARS-CoV-2 receptor binding domain and full-length spike protein, tested negative on three nasal swab PCR tests over a 2-week quarantine period, and subsequently developed a positive PCR test at least 2 weeks after leaving quarantine.[2] We have now investigated whether these SARS-CoV-2-reinfected individuals shed viable virus.

Viral transport media was available from the first and some subsequent PCR-positive tests from 16 (84%) of 19 reinfected participants. Samples were cultured for SARS-CoV-2 in Vero E6 cells expressing *TMPRSS2*[4] in T25 cm² flasks and monitored for cytopathic effect for 4 days followed by a second passage onto fresh cells to allow additional time for virus amplification. Samples were simultaneously titred by plaque assay in Vero E6/*TMPRSS2* cells to quantify the level of detectable infectious virus.

Viable virus was detected in only four (25%) of 16 participants, and only once in each, with titres ranging from 1·7 to 5·5 $\log_{10}$ plaque-forming units per mL. Serology and PCR cycle threshold (Ct) values using the US Food and Drug Administration-authorised Thermo Fisher TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific, Waltham, MA, USA) were compared in those with viable virus detected (shedders) and those without viable virus detected (non-shedders; table; appendix p 2). At the time of positive viral culture, samples from shedders had lower Ct values. Although all participants had detectable SARS-CoV-2 spike IgG at study enrolment, none of the four shedders and only four (33%) of the 12 non-shedders had detectable serum neutralisation activity (limit of detection was 50% inhibitory dilution of 20) in serum at the time of enrolment into the study (methods as described[2]). Only one (25%) of the four who shed virus was symptomatic when viable virus was detected.

All reinfections occurred before December, 2020, when the B.1.1.7 variant was first reported in the USA. Full-length viral genome was recovered from seven (44%) of 16 participants studied by viral transport medium culturing, including all four culture-positive individuals (methods previously described[5]). Although the strains isolated all had the Asp614Gly spike protein mutation and two of the isolates had the Leu18Phe spike protein mutation, they did not have mutations associated with the B.1.1.7, P.1, B.1.351, or other variants of concern as currently defined by the US Centers for Disease Control and Prevention (appendix p 3).

Limitations of our study are the use of virus culture as a proxy for capacity for transmission without direct study of transmission, the small sample size, the absence of specimens available for culturing from all reinfected participants, and the inability to compare potential for transmissibility between those who are infected for the first time and those who are reinfected.

Overall, our findings suggest that about a quarter of young healthy individuals with subsequent SARS-CoV-2 reinfection shed viable virus. Some of these individuals were asymptomatic and could unknowingly transmit SARS-CoV-2 to others.

	Virus culture titre		Difference*
	Positive (n=4)	Negative (n=12)	
Baseline IgG S titre (log10)	2·8 (0·2)	2·9 (0·7)	−0·08 (−0·88 to 0·72), p=0·834
Baseline ID_{50} detected (>20)	0	4 (33%)	−33% (−87 to 21), p=0·207
PCR positive >7 days	3 (75%)	4 (33%)	42% (−20 to 100), p=0·166
Symptomatic	1 (25%)	3 (25%)	−0% (−57 to 57), p=1·000
N gene Ct	16·2 (4·0)	31·9 (6·1)	−15·64 (−22·68 to −8·60), p=0·0003
S gene Ct	17·8 (4·1)	32·3 (6·2)	−14·52 (−21·76 to −7·28), p=0·0007
ORF1ab gene Ct	16·8 (3·9)	31·6 (6·1)	−14·83 (−21·88 to −7·78), p=0·0005

Data are mean (SD) and n (%). Ranges are 95% CIs. When a participant has multiple PCR-positive samples from more than one visit day, only the data from the visit day with the lowest mean Ct values of the three viral genes were included in the data summary. Ct=PCR cycle threshold. ID_{50}=50% inhibitory dilution. *Difference in mean for the continuous variables and difference in percentage for binary variables.

Table: **Baseline serology, clinical features, and SARS-CoV-2 Ct levels in reinfected participants with samples cultured for viable virus**

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For the **study by Letizia AG and colleagues** see **Online/Articles**
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