Prolonged Persistence of SARS-CoV-2 RNA in Body Fluids

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We prospectively assessed 49 coronavirus disease cases in Guangdong, China, to estimate the frequency and duration of detectable severe acute respiratory syndrome coronavirus 2 RNA in human body fluids. The prolonged persistence of virus RNA in various body fluids may guide the clinical diagnosis and prevention of onward virus transmission.

In December 2019, coronavirus disease (COVID-19) caused by a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in Wuhan, China (*1*,*2*). As of April 1, 2020, the virus had expanded to 195 countries, and >820,000 confirmed cases with >40,000 deaths had been recorded (*3*–*6*).

Clinically, the confirmation of SARS-CoV-2 infection relies on detection of virus RNA in various body fluids. The World Health Organization recommends taking upper and lower respiratory samples simultaneously during the acute phase of infection to detect virus RNA. Recent studies reported a persistent shedding of SARS-CoV-2 in upper respiratory and intestinal samples (*7*,*8*). However, the frequency with which SARS-CoV-2 RNA can be detected in body fluids and the period during which it remains detectable are not well understood. A detailed understanding of the dynamics of the early stages of SARS-CoV-2 infection is needed to inform diagnostic testing and prevention interventions, because existing evidence is based only on observations from case reports. We recruited hospitalized patients with COVID-19 from 2 designated provincial emergency hospitals for e merging infectious diseases in Guangdong, China, and tested specimens by real-time reverse transcription PCR (rRT-PCR) to estimate the duration of the detection of SARS-CoV-2 RNA in various body fluids, using an accelerated failure time (AFT)–based modeling study.

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The Study

We recruited 43 patients with mild cases of COVID-19 (22 male, 21 female; median age 43, range 1–70 years) and 6 patients with severe cases (6 male; median age 67, range 46–76 years) for this study. We obtained throat swab, nasopharyngeal swab, sputum, and feces specimens every 3 days for 4 weeks. We tested all specimens by rRT-PCR (Appendix, https://wwwnc.cdc.gov/EID/article/26/8/20-1097-App1.pdf). We used parametric Weibull regression models (AFT) to estimate the time until the loss of SARS-CoV-2 RNA detection in each body fluid and reported findings in medians and 95th percentiles using R software version 3.6.1 with flexsurv, survival, and survminer packages (*9*). We used Lnorm and gamma models as comparisons to evaluate the sensitivity and stability of Weibull regression models. We defined the time until loss of SARS-CoV-2 RNA detection in each fluid as the number of days between the day after illness onset and the day of the first negative rRT-PCR result. For the cases that involved intermittent shedding of SARS-CoV-2, we used the date of the first negative result after the final recorded positive rRT-PCR results. Of the 49 case-patients, 15 were discharged from the hospital after <4 weeks of observation time.

We obtained a total of 490 specimens (32.75% of the designated number of samples, 1,006 missing samples), including 88 throat swab samples (23.53%, 198 missing samples), 62 sputum samples (16.58%,

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Table. Prolonged persistence of SARS-CoV-2 RNA in body fluids from hospitalized patients with coronavirus disease, Guangdong, China*

	Mild cases, n = 43		Severe cases, n = 6	
Specimens	Median (95% CI)	95th percentile (95% CI)	Median (95% CI)	95th percentile (95% CI)
Throat swab	15.6 (11.8–20.7)	32.8 (25.9–42.3)	33.9 (24.2–47.3)	53.9 (39.4–81.7)
Sputum	20.0 (14.1–27.0)	43.7 (33.6–60.4)	30.9 (23.5–39.1)	44.7 (36.3–58.0)
Nasopharyngeal swab	22.7 (18.8–27.5)	46.3 (39.0–55.2)	33.5 (25.7–42.7)	49.4 (38.4–68.5)
Feces	24.5 (21.2–28.3)	45.6 (40.0–52.8)	32.5 (26.3–39.1)	48.9 (41.3–59.7)

*The time until the loss of SARS-CoV-2 RNA detection in each body fluid was estimated by using parametric Weibull regression models. Data are presented as medians and 95th percentiles in days after illness onset. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

312 missing samples), 175 nasopharyngeal swab samples (46.79%, 199 missing samples), and 165 fecal samples (44.12%, 209 missing samples). Of these, 171 specimens tested positive for SARS-CoV-2 RNA by rRT-PCR, including 16 throat swab samples, 38 sputum samples, 89 nasopharyngeal swab samples, and 28 feces samples (Appendix Figure 1). We used Weibull models to estimate the median and the 95th percentile for the time until the loss of SARS-CoV-2 RNA detection in swab, sputum, and fecal samples (Table; Figures 1, 2). The sensitivity and stability evaluation of the Weibull, Lnorm, and gamma models showed no differences among them ($p<0.05$) (Appendix Table, Figures 2, 3).

Conclusions

In this study, we estimated the time for COVID-19 case-patients to clear SARS-CoV-2 RNA in the acute phase of infection through an AFT-based modeling study. We found persistent shedding of virus RNA in nasopharyngeal swab and feces samples. The estimated time until loss of virus RNA detection ranged from 45.6 days for nasopharyngeal swab samples to 46.3 days for feces samples in mild cases and from 48.9 days for nasopharyngeal swab samples to 49.4 days for feces samples in severe cases, which was longer than those of SARS-CoV and MERS-CoV (*10*,*11*). Lan et al. reported positive rRT-PCR results in throat swab sampless from patients who recovered from mild COVID-19 for 50 days at maximum (*8*). Wu et al. found prolonged presence of SARS-CoV-2 viral RNA in fecal samples (*7*). However, we found that the median time for throat samples from mild cases was 15.6 days (95% CI 11.8–20.7 days) and the 95th percentile was 32.8 days (95% CI 25.9–42.3 days). Therefore, detection of virus RNA for mild cases in throat swab samples at the 50th day after illness onset should be a low-probability event, beyond the 95th percentile limit. Similarly, the detection of virus RNA in fecal samples from mild cases was also close to the 95th percentile limit we estimated (45.6 days, 95% CI 40.0-52.8 days).

We found differences in median time until loss of virus RNA detection among respiratory specimen types in mild cases but not in severe cases (Table). We do not believe this finding was linked to the severity of COVID-19, but we had a limited sample size of severe cases in this study. The additional test using Lnorm or gamma models addressed similar phenomena. Nevertheless, the estimated time until the loss of RNA detection in various body fluids in this study was reasonable and was consistent with previous findings in case reports.

Challenges have been raised recently in the molecular diagnosis of COVID-19. Upper respiratory samples show lower positive rates and instable states of confirmation of SARS-CoV-2, whereas lower respiratory samples, such as bronchoalveolar lavage fluid, are suitable specimens for detection of virus RNA (*12*). The probable explanation of discrepant results with our estimates was the irregular operation of sampling in upper respiratory samples in clinics, rather than short-term shedding of virus RNA. In addition, the median duration in archived publications in China was 12.0 days (mean 12.8 days) (*13*), which was shorter than, but close to, our estimate in throat swab samples. This finding was in line with throat swab samples being suggested as a clinical sample for diagnosis of COVID-19 at the early stages of the outbreak in China (National Health Commission of the People's Republic of China, unpub. data, 2020 Jan 15).

Our study has limitations. First, we did not test serum specimens to address RNAemia or serologic trends. The reasons are the findings of extreme low positive rates of RNAemia in our initial study (1 of 49 cases), which does not yield any estimated conclusion. The serologic test was not conducted because reliable IgM/IgG kits were unavailable. Second, virus isolation and tests of specimens' infectivity were not conducted. We focused on estimating the duration of the detection of SARS-CoV-2 RNA in various body fluids among COVID-19 cases but did not imply the existence of infectious virus particles. Third, the number of missing specimens was higher than the initial study designed, attributed mainly to low proportions of purulent sputum production in viral pneumonia cases, as well as low compliance of patients. Fourth, this study may pose selection bias because

modest-sized groups of cases were included. Finally, the prerequisite we assumed was that all COVID-19 cases had SARS-CoV-2 RNA in all sampling specimens at symptom onset, which means that the median and 95th percentile we estimated were shorter than expected because of the uncertainty of incubation time. The time estimated in this study through hospitalized COVID-19 cases may not be generalizable to all infections with SARS-CoV-2, such as asymptomatic cases.

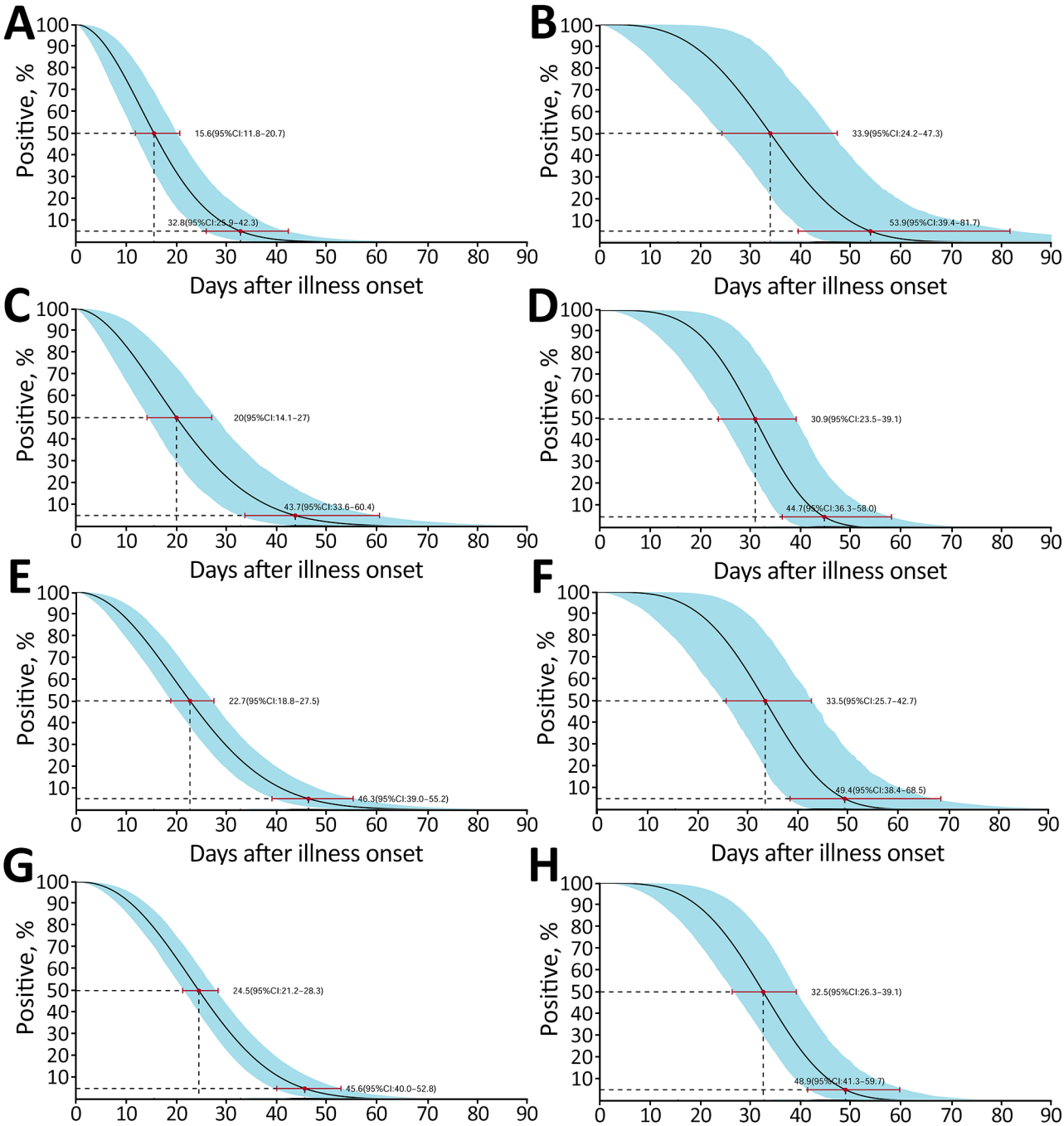

Figure 1. Time until clearance of severe acute respiratory syndrome coronavirus 2 RNA in throat swab, sputum, nasopharyngeal, and feces samples among hospitalized patients with coronavirus disease, as estimated with the use of Weibull regression, Guangdong, China. A, B) Throat swab specimens from patients with mild (A) and severe (B) cases; C, D) sputum samples from patients with mild (C) and severe (D) cases; nasopharyngeal swab samples from patients with mild (E) and severe (F) cases; G, H) feces samples from patients with mild (G) and severe (H) cases. A total of 43 patients with mild and 6 with severe cases were tested. The medians and 95th percentiles of the time until the loss of detection are indicated; error bars and shading indicate 95% CIs.

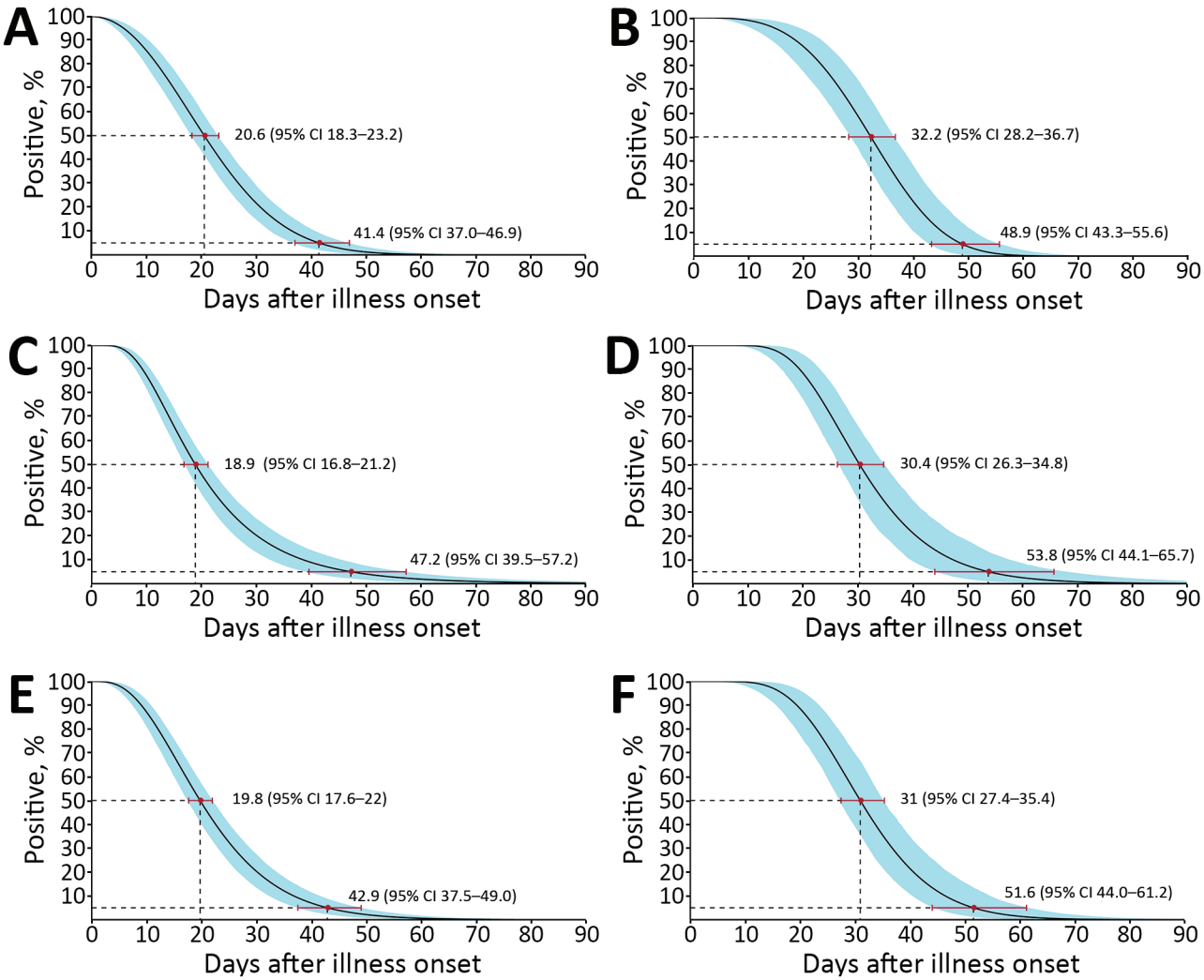

Figure 2. Time until clearance of severe acute respiratory syndrome coronavirus 2 RNA in any clinical specimens of throat swab, sputum, nasopharyngeal swab, or feces samples among hospitalized patients with coronavirus disease, as estimated with the use of Weibull, Lnorm, and gamma regression, Guangdong, China. A, B) Weibull regression for mild (A) and severe (B) cases; C, D) Lnorm regression for mild (C) and severe cases (D); E, F) gamma regression for mild (E) and severe (F) cases. A total of 43 patients with mild and 6 with severe cases were tested. The medians and 95th percentiles of the time until the loss of detection are indicated; error bars and shading indicate 95% CIs.

In conclusion, our results show prolonged persistence of SARS-CoV-2 RNA in hospitalized patients with COVID-19. Health professionals should consider these findings in diagnostic recommendations and prevention measures for COVID-19.

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Appendix

Materials and Methods

Ethics Approval

The novel coronavirus virus disease (COVID-19) was considered part of an ongoing emerging public health outbreak event in China. Data collection and analysis of cases and close contacts were considered exempt from institutional review board approval for center of disease control and prevention in China.

Epidemiology Information Collection

Beginning on January 23, 2020, we prospectively recruited COVID-19 case-patients from the Guangdong Second Provincial General Hospital and the First Hospital of Foshan in Guangdong, China, 2 designated Guangdong provincial emergency hospitals for emerging infectious diseases. All the recruited COVID-19 case-patients were confirmed for SARS-CoV-2 infection through rRT-PCR assay. COVID-19 case-patients transferred to other hospitals after hospitalization in these 2 hospitals were excluded from this study. Cases beyond the observation time because of different dates of illness onset, when they were enrolled or discharged before 4 weeks' observation, were also included for modeling estimation. Demographic information and dates of illness onset were collected from all recruited COVID-19 cases according to the standard epidemiology investigation workflow in those 2 hospitals.

Case Definitions and Criteria for Discharge from Hospital

A clinical suspect case was in a patient who had a travel history to Wuhan or direct contact with patients from Wuhan who had fever or respiratory symptoms, within 14 days before illness onset. A laboratory-confirmed case was defined as a case in a patient with respiratory specimens who tested positive for the SARS-CoV-2 by $\geq$1 of the following 3 methods: isolation of virus or $\geq$2 positive results by rRT-PCR assay for SARS-CoV-2 or a genetic sequence that

matches SARS-CoV-2.The patients were categorized into severe and mild cases depending on whether they were admitted to an intensive care unit or received oxygen supplementation and ventilation treatment during the hospitalization.

The criteria for hospital discharge in China were issued on guidelines for COVID-19 clinical diagnosis and treatment (version I–VI). Patients with COVID-19 who showed absence of clinical symptoms, whose body temperature was normal for >3 days, and whose lung computed tomography improved notably, without any acute manifestations such as exudation, and had with 2 serial negative RT-PCR test results (24 h interval) could be discharged from the hospital.

Specimen Collection and Storage

Specimen collection from COVID-19 case-patients was conducted in consultation with a healthcare provider. For nasopharyngeal specimens, a swab was inserted into the nostril parallel to the palate to a depth equal to the distance from the nostrils to the outer opening of the ear. The swab was left in place for several seconds to absorb secretions and then was slowly removed with rotation. For throat samples, the posterior pharynx was swabbed, avoiding the tongue. For sputum samples, the patient was asked to rinse the mouth with water and then expectorate a deep cough sputum directly into a sterile, leakproof, screw-cap collection cup. Feces samples were also collected in screw-cap collection cups. Specimens were immediately stored at 2–8°C and transported under the same conditions to the Guangdong Provincial Center for Disease Control and Prevention for viral RNA extraction and molecular testing. Residual specimens were stored at –70°C or below.

Nucleic Acid Extraction and rRT-PCR

The swab samples were vortexed in 2.5 mL of commercial viral transport media and 200 μL was recovered for RNA extraction. For fecal samples, ≈10 g of feces were added to a tube with 10 mL of phosphate-buffered saline (PBS) and glass beads. After vortexing at 2000 rpm for 10 min, the same volume (200 μL) of supernatant was recovered for RNA extraction. For sputum samples, the sputum was added to a tube with 5 mL of PBS and glass beads. After vortexing at 2000 rpm for 10 min, we recovered the same volume of supernatant for RNA extraction.

We extracted total RNA using a prefilled viral total NA kit-Flex (Fisher Scientific, Labserv, Cat.No. KFRPF-805296; https://www.fishersci.com) following manufacturer's instructions. All samples were lysed, and the nucleic acids bound to the surface of magnetic

beads. The beads were washed with buffers to remove residual proteins and contaminants and the purified nucleic acids were eluted with 50 μL of nuclease free water for subsequent testing.

A commercial rRT-PCR assay kit targeting the ORF1ab and N genes was used to detect SARS-CoV-2 RNA (DaAn Gene, Guangzhou, China, cat. no. DA0931; http://en.daangene.com). Amplification was performed on an Applied Biosystems 7500 Real-Time PCR System (ThermoFisher Scientific, https://www.thermofisher.com) as follows: 50°C for 15 min, 95°C for 15 min, followed by 45 cycles of 94°C for 15s and 55°C for 45s. Specimens were considered positive for SARS-CoV-2 RNA if both ORF1ab and N gene target amplification curves were generated within 40 cycles.

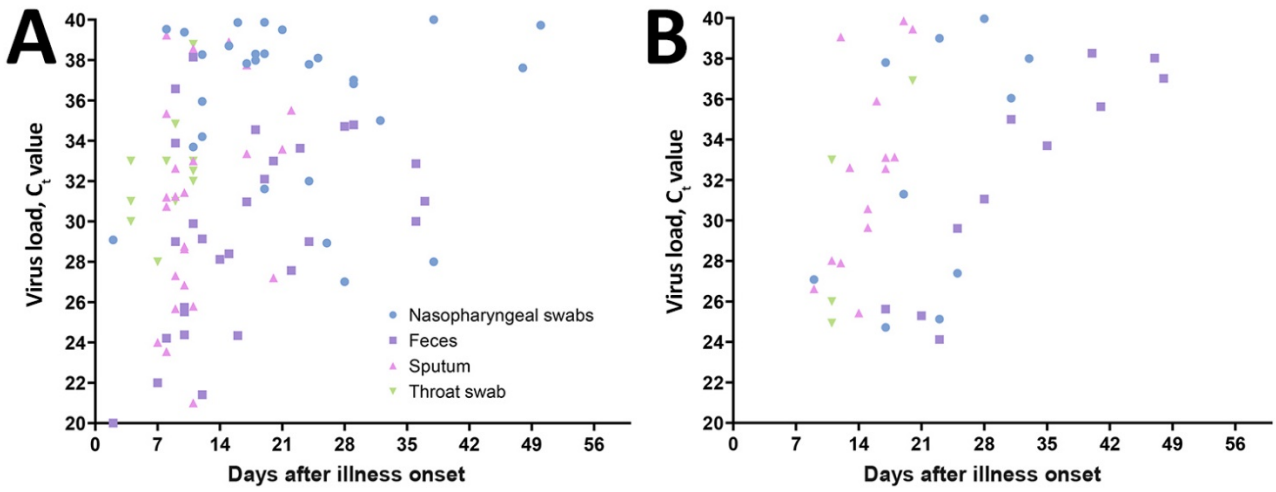
Modeling Estimates

We fit separate parametric accelerated failure time (AFT) survival models (Weibull, Lnorm, and gamma) to the outcomes of the time to loss of RNA detection in throat swabs, nasopharyngeal swabs, sputum samples, and feces specimens. The outcome of time to loss of RNA detection in each fluid was defined as the days until the first negative RT-PCR result. For those shedding intermittently, we used the first negative result after the final recorded RT-PCR-positive test result. Survival time estimates at given quantiles of cumulative survival (50th and 95th percentiles) by using standard maximum-likelihood estimation approaches.

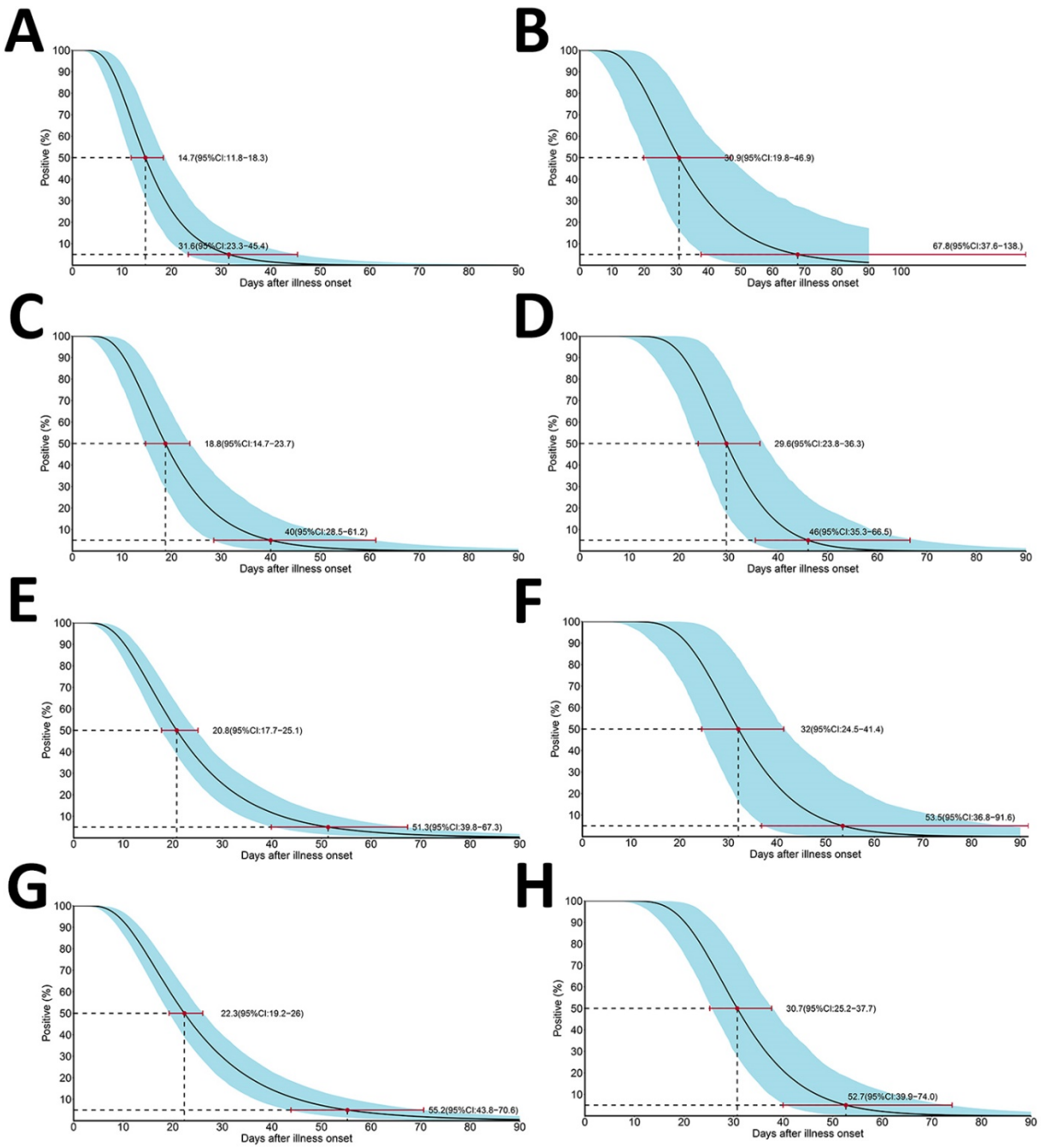
Appendix Table. Prolonged persistence of SARS-CoV-2 in body fluids.

		Mild cases (n = 43)		Severe cases (n = 6)	
Model	Specimens	Median (95% CI)	95th percentile (95% CI)	Median (95% CI)	95th percentile (95% CI)
Gamma	Throat swab	15.3 (12.0–19.1)	31.1 (24.0–40.9)	31.9 (20.7–46.2)	61.3 (39.6–102.0)
	Sputum	19.6 (14.9–25.7)	40.8 (29.3–56.4)	29.9 (24.1–37.2)	45.4 (34.8–61.9)
	Nasopharyngeal swab	21.8 (18.4–25.5)	47.4 (39.3–57.3)	32.5 (25.5–40.9)	51.6 (37.3–76.0)
	Feces	23.3 (20.2–26.9)	49.0 (41.3–59.3)	31.3 (25.7–37.8)	51.0 (40.8–66.2)
Lnorm	Throat swab	14.7 (11.8–18.3)	31.6 (23.3–45.4)	30.9 (19.8–46.9)	67.8 (37.6–138.0)
	Sputum	18.8 (14.7–23.7)	40.0 (28.5–61.2)	29.6 (23.8–36.3)	46.0 (35.3–66.5)
	Nasopharyngeal swab	20.8 (17.7–25.1)	51.3 (39.8–67.3)	32.0 (24.5–41.4)	53.5 (36.8–91.6)
	Feces	22.3 (19.2–26.0)	55.2 (43.8–70.6)	30.7 (25.2–37.7)	52.7 (39.9–74.0)

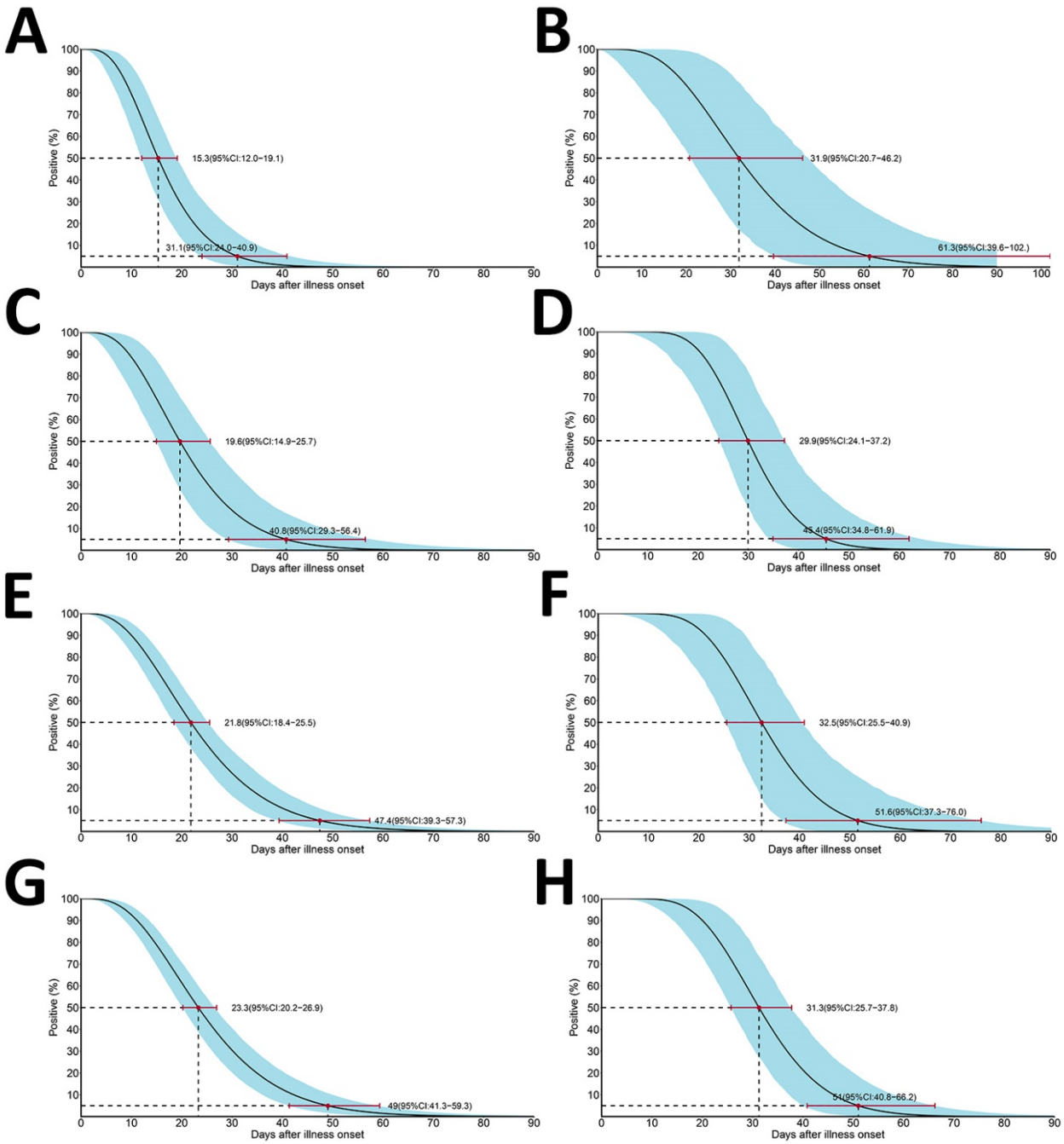
*The time until the loss of SARS-CoV-2 RNA detection in each body fluid was estimated by using parametric gamma and Lnorm regression models. The data are presented as medians and 95th percentiles since days after illness onset.



Appendix Figure 1. Specimens were considered positive if target amplification was detected within 40 amplification cycles. Viral loads were expressed as the cycle threshold (C_t) value of rRT-PCR. Results are presented for A) mild and B) severe COVID-19 cases in throat swabs, sputum, nasopharyngeal swabs, and feces samples.



Appendix Figure 2. Time until the clearance of SARS-CoV-2 RNA in throat swabs, sputum, nasopharyngeal swabs, and feces using an Lnorm regression model. Shown are the time until the loss of SARS-CoV-2 RNA detection after the onset of symptoms in throat swabs (A, mild cases; B, severe cases), sputum (C, mild cases; D, severe cases), nasopharyngeal swabs (E, mild cases; F, severe cases), and feces (G, mild cases; H, severe cases) from 43 mild and 6 severe COVID-19 cases in hospitalized patients. The medians and 95th percentiles of the time until the loss of detection is shown in each panel with 95% confidence intervals (blue shading).



Appendix Figure 3. Time until the clearance of SARS-CoV-2 RNA in throat swabs, sputum, nasopharyngeal swabs, and feces by using a gamma regression model. Shown are the time until the loss of SARS-CoV-2 RNA detection after the onset of symptoms in throat swabs (A, mild cases; B, severe cases), sputum (C, mild cases; D, severe cases), nasopharyngeal swabs (E, mild cases; F, severe cases), and feces (G, mild cases; H, severe cases) from 43 mild and 6 severe COVID-19 cases in hospitalized patients. The medians and 95th percentiles of the time until the loss of detection is shown in each panel with 95% confidence intervals (blue shading).