Title

No Evidence of SARS-CoV-2 Seminal Shedding Despite SARS-CoV-2 Persistence in the Upper Respiratory Tract

Journal

Open Forum Infectious Diseases, 7(8)

ISSN

2328-8957

Authors

Rawlings, Stephen A

Ignacio, Caroline

Porrachia, Magali

et al.

Publication Date

2020-08-01

DOI

10.1093/ofid/ofaa325

Peer reviewed

Open Forum Infectious Diseases

BRIEF REPORT

No Evidence of SARS-CoV-2 Seminal Shedding Despite SARS-CoV-2 Persistence in the Upper Respiratory Tract

Stephen A. Rawlings,[1] Caroline Ignacio,[1] Magali Porrachia,[1] Pinyi Du,[1] Davey M. Smith,[1,a] and Antoine Chaillon[1,a]

[1]Division of Infectious Diseases and Global Public Health, University of California San Diego, San Diego, California, USA

RNA viruses (eg, Zika, Ebola, HIV) are often shed in male genital secretions. We evaluated the presence and level of SARS-CoV-2 RNA in semen, nasal secretion, and saliva collected after confirmed infection. SARS-CoV-2 RNA was not detected in semen 6–17 days after the onset of symptoms despite concomitant shedding in oral secretions.

Keywords. SARS-CoV-2; semen; shedding; transmission.

While it is known that severe acute respiratory coronavirus 2 (SARS-CoV-2) infection often starts with 1–2 days of asymptomatic shedding before a person gets sick, there are limited data about the biological source and duration of viral shedding after symptom resolution in recently infected individuals. Besides respiratory droplets, SARS-CoV-2 RNA has been isolated in other biological samples including urine, blood, feces, and saliva [1, 2]. The expression of SARS-CoV-2 receptor angiotensin 2–converting enzyme (ACE2) across multiple tissues also suggests that SARS-CoV-2 could be found in other tissues and body fluids [3], including in the testis and male genital tract [4, 5]. Very few studies exist on the presence of SARS-CoV-2 RNA in semen, and these studies have been limited by considerable time between semen sampling and diagnosis of active infection. In particular, when semen has been examined ~4–6 weeks after SARS-CoV-2 infection, the virus was not detected, and a case report of a single male acutely infected revealed no SARS-CoV-2 in seminal fluid [6–8]. Whether SARS-CoV-2 can be detected in semen during the acute phase of SARS-CoV-2 infection when the virus is concomitantly present in nasal and/or oral secretions remains a concern. Here, we evaluated the presence and level of SARS-CoV-2 in paired semen, nasal secretion, and saliva samples collected in the short and medium term after confirmed SARS-CoV-2 symptomatic infections.

METHODS

Patient Consent Statement

All participants provided informed, written consent. This study was conducted under a protocol for collecting samples from persons with known SARS-CoV-2 infection approved by the Institutional Review Board of University of California San Diego.

Study Cohort and Sampling

Men who had been diagnosed with SARS-CoV-2 based on a combination of medical history, symptoms, and the presence of SARS-CoV-2 RNA in the upper respiratory tract were invited to enroll in this study. All participants were outpatients at the time of enrollment and sample collection. They were instructed to self-collect saliva (passive drool), nasal swab, and semen by masturbation without lubricant after 24 hours of abstinence. Samples were collected within 1–3 weeks after the onset of symptoms.

Processing of Semen Samples

Semen was processed as in Butler et al. [9]. Briefly, viral transport medium (2 mL of RPMI 1640 with 2 mMol/L of glutamine and 10% fetal bovine serum [FBS], with the addition of 100 U/mL of penicillin, 100 µL/mL of streptomycin, and 200 U/mL of nystatin) was added to seminal samples at collection. Seminal plasma was separated from seminal cells by centrifugation at 700 × *g* for 12 minutes within 4 hours of collection and stored at −80°C and −150°C, as previously described [9].

SARS-CoV-2 RNA Extraction

RNA was extracted from seminal plasma, saliva, and viral transport media that contained the nasopharyngeal (NP) swab; 140 µL of each type of sample was extracted for RNA using Qiagen's QIAamp Viral RNA mini kit (cat# 52904) according to the manufacturer's recommendation. cDNA from SARS-CoV-2 RNA was generated using the Bio-Rad One-Step RT-ddPCR Advanced Kit for Probes (cat# 186–4021).

SARS-CoV-2 RNA Detection and Quantification

Qualitative tests for SARS-CoV-2 were performed on the first collected nasal/NP specimen for diagnostic confirmation using

Received 13 May 2020; editorial decision 24 July 2020; accepted 28 July 2020.

[a]Equal contribution

Correspondence: Antoine Chaillon, MD, PhD, Center For AIDS Research (CFAR), Division of Infectious Diseases, UCSD & VA San Diego Healthcare System, Stein Clinical Research Building #325, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0679 (achaillon@ucsd.edu).

Open Forum Infectious Diseases®

DOI: 10.1093/ofid/ofaa325

the FluxErgy platform (Irvine, CA, USA), which is currently available as a Research Use Only (RUO) or Investigational Use Only (IUO) device for the development of new diagnostic products.

Quantitative measure of SARS-CoV-2 in saliva and semen was performed using digital droplet polymerase chain reaction (ddPCR; BIO-RAD QX200 Droplet Reader). Copy numbers were calculated as the mean of 3 replicates. Briefly, we have adapted our validated protocol [10] to quantify SARS-CoV-2 in various samples following the Centers for Disease Control and Prevention recommendations and the 2019-nCoV Real-Time RT-PCR Diagnostic Panel targeting the virus nucleocapsid (N) and the ORF gene [11]. Based on testing and dilutions with known positive and negative clinical samples (data not shown), the current level of detection is 0.05 copies/μL.

RESULTS

A total of 6 participants aged 28–45 years were enrolled in this study (Supplementary Table 1). All initially presented with clinical symptoms compatible with SARS-CoV-2 and/or recent history of close contact with a confirmed case. Symptoms included cough, shortness of breath, fever, myalgia, fatigue, anosmia, headache, anorexia, and diarrhea.

Before enrollment, 5/6 participants tested positive for SARS-CoV-2 RNA on NP swab samples collected within 1–3 days following the onset of symptoms. ID16 was not initially tested by his clinical team, but the diagnosis was supported by both clinical symptoms and recent close contact with a confirmed case. Upon enrollment in the study, the diagnosis of active SARS-CoV-2 infection was confirmed by positive PCR on NP swab on day 6 post–symptom onset. The clinical conditions of all participants improved, with complete resolution of initial symptoms, within 1–3 weeks from the onset.

Paired saliva and semen samples were collected a mean of 12 days (6–17 days) after the onset of symptoms, and ddPCR was performed to quantify the SARS-CoV-2 level in all samples. Half of the participants also had research nasal swabs performed. All 6 semen samples were negative for SARS CoV-2 (≤0.03 copies/μL), while SARS-CoV-2 was still detected in all saliva samples (6 participants) and all research nasal swabs (3 participants). Saliva levels of virus were quantified and varied from 0.05 to 679 copies/μL of input saliva (Figure 1, Table 1).

DISCUSSION

The temporal dynamic of viral shedding and transmissibility of SARS-CoV-2 remains a major concern to control the spread of the virus and directly impacts control measures such as isolation, contact tracing, and enhanced hygiene or use of face masks for symptomatic persons. Recent studies have shown persistant viral detection in the upper respiratory tract up to 37 days after the onset of symptoms [12]. ACE2 expression across tissues and body fluid—including seminal fluid—suggests possible extrarespiratory transmission routes [13]. Here, we investigated the presence of SARS-CoV-2 in the semen of men in whom virus was demonstrably present in the respiratory tract. We found no evidence of SARS-CoV-2 in semen collected 6–17 days after the onset of symptoms despite all men having concomitant shedding of virus in oral secretions up to 792 copies/μL. Though the study is small in number, it adds to available literature that the male genital tract does not appear to be a site where SARS-CoV-2 is shed in either the acute or late phase of infection. Identifying whether SARS-CoV-2 can

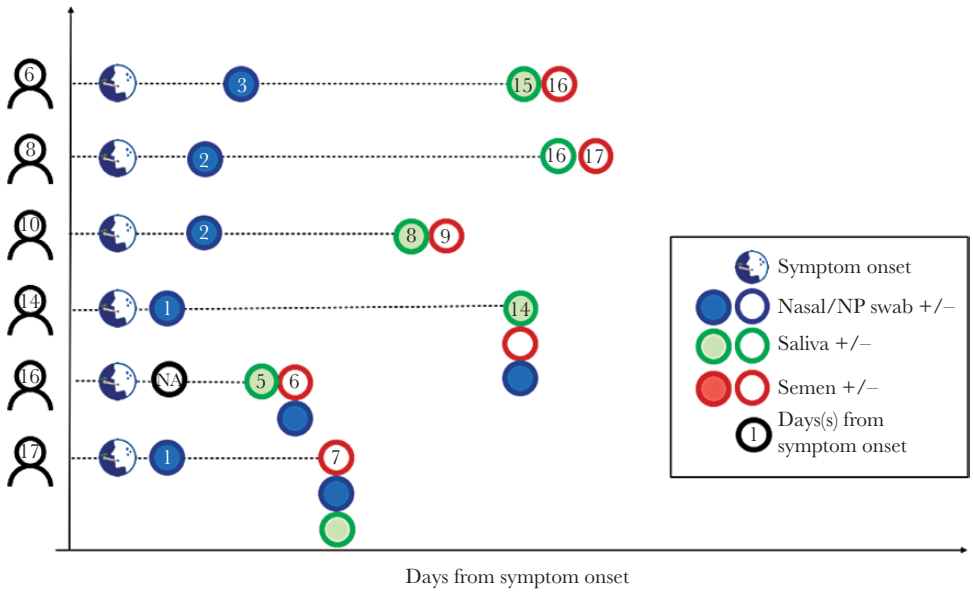

Figure 1. Sampling and SARS-CoV-2 testing history. See Table 1 for quantitative measures via digital droplet polymerase chain reaction. Abbreviation: NP, nasopharyngeal.

Table 1. Digital Droplet PCR Measures of SARS-CoV-2 in Paired Saliva, Nasal Secretion, and Semen

Participant ID	Copies/μL of Saliva (Days From Onset)	Repeat, Experimental Nasal Swab (Days From Onset)	Copies/μL of Semen (Days From Onset)
ID6	0.12 (15)	Not done	≤0.03 (16)
ID8	0.05 (16)		≤0.03 (17)
ID10	63.9 (6)		≤0.03 (9)
ID14	679 (14)	Positive (14)	≤0.03 (14)
ID16	11.8 (5)	Positive (6)	≤0.03 (6)
ID17	12.7 (7)	Positive (7)	≤0.03 (7)

Abbreviation: PCR, polymerase chain reaction.

be shed in semen has implications for public health, as all predominant modes of transmission (ie, droplet, sexual contact, airborne, fomite, etc.) need to be identified in order to help curb the spread of the virus. With the small number of participants in this study, it is difficult to quantify the absolute probably of spread in a large population, but the complete absence of virus using ultrasensitive methods suggests that shedding of the virus in semen is certainly not common. A larger study would be needed to demonstrate and quantify rare events of shedding.

Whether SARS-CoV-2 can be detected in the semen at the very early phase of infection or during the incubation period when it is present in the upper respiratory tract [12] would require further investigation, but collective evidence [6–8] and our study suggest that SARS-CoV-2 is not present in semen.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

Fluxergy, Inc. The authors thank the participants and their families.

Author contributions. S.A.R. performed study procedures, analyzed data, and reviewed the manuscript. B.S., L.L., M.P., and C.I. performed study procedures and reviewed the manuscript. A.C. and D.S. analyzed data and wrote the manuscript.

Financial support. This work was supported by the John and Mary Tu Foundation and the Translational Virology Core of the San Diego Center for AIDS Research (CFAR) grant number AI036214, a National Institutes of Health–funded program. S.A.R. was supported by the National Institutes of Health (grant number 5T32AI007384). A.C. was supported by the National Institutes of Health (grant number AI131971) and the University of California Office of the President (UCOP R00RG2725).

Potential conflicts of interest. A.C., S.A.R., B.S., L.L., M.P., and C.I. have no conflicts. D.S. is a consultant for FluxErgy, Bayer, AIDS Healthcare Foundation and Arena Pharmaceuticals. All other authors report no conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Ling Y, Xu S-B, Lin Y-X, et al. Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients. Chin Med J **2020**; 133:1039–43.
2. Xie C, Jiang L, Huang G, et al. Comparison of different samples for 2019 novel coronavirus detection by nucleic acid amplification tests. Int J Infect Dis **2020**; 93:264–7.
3. Xu H, Zhong L, Deng J, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci **2020**; 12:8.
4. Wang Z, Xu X. scRNA-seq profiling of human testes reveals the presence of the ACE2 receptor, a target for SARS-CoV-2 infection in spermatogonia, leydig and sertoli cells. Cells **2020**; 9.
5. Fan C, Li K, Ding Y, Lu WL, Wang J. ACE2 expression in kidney and testis may cause kidney and testis damage after 2019-nCoV infection. medRxiv 20022418 [Preprint]. 13 February **2020**. Available at: **https://doi.org/10.1101/2020.02.12.20022418**. Accessed May 5, 2020.
6. Song C, Wang Y, Li W, et al. Absence of 2019 novel coronavirus in semen and testes of COVID-19 patients†. Biol Reprod. **2020**; 103:4–6.
7. Paoli D, Pallotti F, Colangelo S, et al. Study of SARS-CoV-2 in semen and urine samples of a volunteer with positive naso-pharyngeal swab. J Endocrinol Invest. **2020**; 1–4.
8. Pan F, Xiao X, Guo J, et al. No evidence of severe acute respiratory syndrome-coronavirus 2 in semen of males recovering from coronavirus disease 2019. Fertil Steril. **2020**; 113:1135–1139.
9. Butler DM, Delport W, Kosakovsky Pond SL, et al. The origins of sexually transmitted HIV among men who have sex with men. Sci Transl Med **2010**; 2:18re1.
10. Strain MC, Lada SM, Luong T, et al. Highly precise measurement of HIV DNA by droplet digital PCR. PLoS One **2013**; 8:e55943.
11. Centers for Disease Control and Prevention. CDC 2019-novel coronavirus (2019-nCoV) real-time RT-PCR diagnostic panel. Available at: https://www.cdc.gov/coronavirus/2019-ncov/lab/index.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fcoronavirus%2F2019-ncov%2Flab%2Frt-pcr-detection-instructions.html. Accessed May 5, 2020.
12. He X, Lau EHY, Wu P, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med **2020**; 26:672–5.
13. Chen Y, Guo Y, Pan Y, Zhao ZJ. Structure analysis of the receptor binding of 2019-nCoV. Biochem Biophys Res Comm **2020**; 525:135–40.