

We declare no competing interests.

*Kristan A Schneider, Martin Eichner

kristan.schneider@hs-mittweida.de

Department of Applied Computer Sciences and Biosciences, Hochschule Mittweida, Mittweida 09648, Germany (KAS); Institute for Clinical Epidemiology and Applied Biometrics, University of Tübingen, Tübingen, Germany (ME); Epimos, Bischofsheim in der Rhön, Germany (ME)

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Monkeypox virus isolation from a semen sample collected in the early phase of infection in a patient with prolonged seminal viral shedding



The unexpected increase in human monkeypox cases in non-endemic countries that began in May, 2022, is raising concerns of a novel global infectious threat. Since the first human case in 1970 in the Democratic Republic of the Congo, the virus has become endemic in several countries in central and western Africa.¹ Imported cases have been sporadically reported outside Africa (in England, the USA, Singapore, and Israel), with the majority of cases associated with travellers returning from endemic countries, or due to nosocomial contact or contact with infected imported rodents.^{2,3} As of July 22, 2022, 16 016 laboratory-confirmed monkeypox cases have been reported from 75 countries worldwide, and the WHO Director-General has declared the escalating global monkeypox outbreak to be a public health emergency of international concern.⁴ The vast majority of cases have been reported in Europe and other non-endemic countries, mostly diagnosed in young men, self-identifying as men who have sex with men (MSM). Monkeypox virus transmission might occur through close contact of mucosa or non-intact skin with infectious material, or large respiratory droplets during prolonged face-to-face contact.⁵ Whether monkeypox virus can be sexually transmitted via genital fluids remains under investigation. Monkeypox virus transmission during sexual intercourse has been documented in the UK in two men with no travel history

to endemic countries and evidenced by the temporal association of symptoms with sexual contact and the location of primary lesion sites matching those of sexual contact.⁶ Viral DNA detection in semen samples has been reported in three cases in Italy and subsequently in two patients with monkeypox in Germany.^{7,8} Furthermore, monkeypox DNA was detected in the seminal fluid of 29 (91%) of 32 people affected by monkeypox in a large case series on the 2022 global outbreak.⁹ However, to date, no evidence is available on the infectiousness of monkeypox virus in semen. Therefore, we investigated viral shedding in longitudinal semen samples collected 5–19 days after symptom onset from one confirmed monkeypox virus case diagnosed at the National Institute for Infectious Diseases ‘Lazzaro Spallanzani’ (Rome, Italy; appendix p 1).

The patient was a 39-year-old man, who travelled in Austria during the first 2 weeks of May, 2022. He self-identified as an MSM and sex worker and reported condomless sexual intercourse with several male partners during the previous month. The patient was HIV-infected, treated with dolutegravir and lamivudine, with viral suppression and immune recovery, and reported a history of sexually transmitted infections. He was admitted to the hospital 5 days after symptom onset. His symptoms included fever, followed by the appearance of clustered itchy papular lesions in the anal region and

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| | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 | Day 11 | Day 13 | Day 14 | Day 15 | Day 16 | Day 17 | Day 19 |
|---------------------|-----------------|-----------------|-----------------|-----------------|----------|----------|----------|----------|-----------------|-----------------|----------|-----------------|-----------------|
| Plasma | NA | NA | NA | Positive (34.5) | NA | Negative | NA | Negative | NA | Negative | Negative | Negative | Negative |
| Urine | NA | NA | Negative | NA | Negative | NA | Negative | Negative | NA | Negative | NA | NA | Negative |
| Semen | Positive (28.0) | Positive (29.3) | Positive (27.8) | NA | NA | NA | NA | NA | Positive (34.3) | Positive (35.6) | NA | Positive (38.7) | Positive (40.6) |
| Rash or skin lesion | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Negative |

Quantification cycle values are indicated in brackets after positive results. The cutoff cycle threshold is 45, thresholds of 42 or higher are retested for confirmation. Negative indicates no detection of monkeypox virus DNA or absence of rash or skin lesions. Positive indicates detection of monkeypox virus DNA or presence of rash or skin lesions. NA=not available.

Table: Timeline of monkeypox virus DNA detection in plasma, urine, and semen samples with increasing days from symptom onset

single lesions on the head, thorax, legs, arms, hand, and penis. The patient reported one dose of smallpox vaccination during childhood, more than 30 years earlier. He did not receive any current treatment for monkeypox virus infection.

Monkeypox virus infection was confirmed by real-time PCR on a skin lesion swab (quantification cycle [Cq] 18.9) and scab (Cq 21.4) collected on day 5 after symptom onset. The virus was successfully isolated in vitro from a swab of a skin lesion on the head (appendix p 2). Plasma, urine, and semen samples were longitudinally collected to monitor the duration of viral shedding (table). Monkeypox virus DNA was detected in plasma collected on day 8 after symptom onset only. Urine samples were negative. Monkeypox virus DNA was detected in all semen samples tested during the period of observation (Cq range 27.8–40.6). Semen collected on day 6 after symptom onset was inoculated in Vero E6 cells (ATCC; Manassas VA, USA). Clear cytopathic effect was observed 48 h after the inoculum and monkeypox virus replication was confirmed by real-time PCR on DNA purified from cell growth medium collected after 48 h, 72 h, and 96 h. Notably, on day 6 after symptom onset, anti-monkeypox virus IgG antibodies (1:80) were detected by immunofluorescence assay in the patient’s serum but IgM was not detected. A serum sample on day 8 after symptom onset was positive for both IgG (1:320) and IgM (1:20). This early detection of specific IgG might be related to a possible cross-reaction from childhood smallpox vaccination. However, the fast generation of IgG compared with IgM might not exclude unconventional antibody kinetics, or the presence of low IgM levels during the acute phase of infection, with a titre near the detection limit of the method. Overall, our findings support that prolonged shedding of monkeypox virus DNA can occur in the semen of infected patients for weeks after symptoms onset, and show that semen

collected in the acute phase of infection (day 6 after symptom onset) might contain a replication-competent virus and represent a potential source of infection. Viral particles in semen might derive from passive diffusion from blood, urine, or genital lesions (eg, exfoliated epithelial cells) or local genital replication.¹⁰ Whether infectious monkeypox virus found in semen could be associated with seminal cells or if viral replication occurs in the genital tract remains to be established. In the case discussed herein, the isolation of live replication-competent monkeypox virus from semen, and prolonged viral DNA shedding, even at low viral copies, might hint at a possible genital reservoir. No monkeypox virus DNA was detected in urine and blood samples, suggesting absence of semen cross-contamination from other potential sources. Our patient was an HIV-infected, viro-immunological responder, thus we cannot entirely exclude the possibility of an effect of HIV-associated chronic immune dysregulation on prolonged monkeypox virus shedding in semen. At the time of writing, we detected monkeypox virus DNA in semen samples from 11 (79%) of 14 patients, and live and replication-competent virus was isolated from the positive seminal fluid (Cq 22.7) of a second patient with HIV.

In our opinion, the case discussed herein supports that transmission of monkeypox virus during sexual activity might be a viable and recognised route, especially in the current 2022 outbreak of disease. Further studies on the viral tropism for the genital tract and data on the frequency of virus detection and duration of replicative monkeypox virus shedding in the seminal fluid, also in patients who do not have HIV, are crucial to better understand the viral pathogenesis and the potential role of semen-driven transmission in the spreading of monkeypox infection and disease burden.

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Daniele Lapa, Fabrizio Carletti, Valentina Mazzotta, Giulia Matusali, Carmela Pinnetti, Silvia Meschi, Roberta Gagliardini, *Francesca Colavita, Annalisa Mondì, Claudia Minosse, Laura Scorzoloni, Stefania Cicalini, Gaetano Maffongelli, Eliana Specchiarello, Marta Camici, Aurora Bettini, Francesco Baldini, Massimo Francalancia, Klizia Mizzoni, Anna Rosa Garbuglia, Emanuele Nicastrì, Enrico Girardi, Andrea Antinori, Francesco Vaia, Fabrizio Maggi, on behalf of the INMI Monkeypox Study Group

francesca.colavita@inmi.it

Laboratory of Virology (DL, Fca, GMat, SM, FCo, CM, ES, AB, MF, KM, ARG, FM), Clinical and Research Department (VM, CP, RG, AM, LS, SC, GMaf, MC, FB, EN, AA), Scientific Direction (EG), and General Direction (FV), National Institute for Infectious Diseases ‘Lazzaro Spallanzani’ (IRCCS), Rome, Italy

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Monkeypox: how will we know if the treatments work?

Clinical trials of treatments are a priority for emerging infectious disease outbreaks. When we design trials, fit-for-purpose endpoints are crucial, as these will inform decisions on case management, regulatory approval, and priority for public health funding and interventions.

Monkeypox highlights the difficulties in designing primary endpoints for emerging diseases. Globally, we have a limited understanding of what typical monkeypox is—the common and most severe symptoms, the symptoms that cause most distress to patients, the duration of infectivity, and potential complications. Furthermore, patterns of disease might vary, both between individuals and between different clades of virus. Descriptions of clade I disease emphasise disseminated rash and describe a mortality of around 10%.¹ Experience of clade IIb or III disease outside Africa suggests a predominance of genitourinary and perianal lesions,² with new complications (such as proctitis),²

and there have been no deaths caused by these clades in the current outbreak. Although case ascertainment bias cannot be excluded, causes for milder disease need to be established and might be linked to the way the virus is being transmitted. Our primary motivations for treating monkeypox vary depending on severity and risk of transmission and, therefore, might shift focus between symptom relief, preventing complications, shortening the duration of patient isolation, or preventing spread of disease.

Our understanding of a disease grows with the number of cases and, in the field of emerging infections, by use of standardised clinical characterisation and biological sampling protocols.³ However, waiting for optimal clinical understanding before starting a trial is impractical—many outbreaks are short-lived (especially when working within the geographical borders of regulatory agencies) and we perpetually risk being too late, with the outbreak being declared over before the trial recruits.⁴



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