Elsevier has created a [Monkeypox Information Center](#) in response to the declared public health emergency of international concern, with free information in English on the monkeypox virus. The Monkeypox Information Center is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its monkeypox related research that is available on the Monkeypox Information Center - including this research content - immediately available in publicly funded repositories, with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the Monkeypox Information Center remains active.
Short communication

Clinical characteristics and comparison of longitudinal qPCR results from different specimen types in a cohort of ambulatory and hospitalized patients infected with monkeypox virus.

Dominik Nörz, Thomas Theo Brehm, Hui Ting Tang, Ilka Grewe, Lennart Hermanussen, Hanna Matthews, Julia Pestel, Olaf Degen, Thomas Günther, Adam Grundhoff, Nicole Fischer, Marylyn M. Addo, Sabine Jordan, Sandra Hertling, Stephan Unger, Guido Schäfer, Knud Schewe, Christian Hoffmann, Martin Aepfelbacher, Susanne Pfefferle, Julian Schulze zur Wiesch, Stefan Schmiedel, Marc Lütgehetmann

* Corresponding author.

E-mail addresses: d.noerz@uke.de (D. Nörz), mluetgeh@uke.de (M. Lütgehetmann).

1 Authors contributed equally

https://doi.org/10.1016/j.jcv.2022.105254

Received 9 July 2022; Received in revised form 3 August 2022; Accepted 6 August 2022

Available online 11 August 2022

1386-6532/© 2022 Elsevier B.V. All rights reserved.
1. Introduction

As of July 2022, 7553 confirmed cases of monkeypox have been reported in previously non-endemic countries worldwide [1]. In contrast to previous clusters, the ongoing outbreak appears to be driven exclusively by human-to-human transmission, with the majority of current cases reported among men who have sex with men (MSM) [2–7]. Clinical presentation has been highly variable with patients often lacking the classically described symptoms such as fever and lymphadenopathy [8]. Lesions may be scarce, located only in the anogenital area or even limited to a single lesion [8]. Furthermore, recent reports suggest the existence of asymptomatic infections [9].

Typical presentation entails the risk of missing cases and may also represent a challenge for diagnostics. Current WHO guidance recommends collection of two swab samples from skin lesions, while also encouraging additional oropharyngeal swabs [10]. Recent studies suggest that monkeypox virus-DNA is readily detectable in respiratory specimens and blood, though there is still insufficient data on the reliability and viral load dynamics in these specimen types throughout the course of disease [11].

In this study, we provide longitudinal quantitative PCR-data for different specimen types from a well-characterized cohort of hospitalized patients, and outpatients with confirmed monkeypox virus infection, associated with the current outbreak according to phylogenomic characterization of whole-genome sequences [12]. Further, we were able to confirm infectivity by successful viral culture in initial lesion swab samples of two patients.

2. Material and methods

2.1. Sample collection

In total, 16 patients diagnosed with monkeypox virus infection at the University Medical Center Hamburg-Eppendorf (UKE) were included in this study. Of these, 5 were hospitalized at the UKE, allowing for longitudinal viral load measurements. A further 5 were outpatients at the UKE and patient meta-data and clinical characteristics were available. 6 were external outpatients and only initial viral load data was available. For a study overview see supplementary figure 1.

Lesion swabs and oropharyngeal swabs were performed using eSwab collection kit (Copan, Italy) or VTM collection kit (Citotest, Jiangsu, China). All samples were aliquoted and inactivated by adding ≤40% guanidine hydrochloride solution in Tris–HCl prior to processing for molecular diagnostics.

The study was conducted according to the guidelines of the Declaration of Helsinki. The use of patient data and anonymized samples was approved by the ethics committee of the Medical Council of Hamburg (PV 7298 and PV5626) and additional written consent from patients was obtained for images presented in this study.

2.2. Laboratory methods

Molecular diagnostics, next generation sequencing, immunofluorescence tests and viral culture were performed as described previously [13–17]. Methods are described in more detail in supplementary material 1.

3. Results

3.1. Patient characteristics

The first ten patients presenting with monkeypox virus infection at our center (until June 30th, 2022) were male and identified as MSM. While all patients presented with skin lesions, oral lesions were observed

---

Table 1

Clinical characteristics of hospitalized patients and outpatients. All patients were male and between 20 and 40 years old. Two patients were HIV-positive and currently under antiretroviral therapy (ART), four HIV-negative patients are taking Pre-exposition prophylaxis (PrEP) and four HIV-negative patients are not taking any prophylaxis. In all cases lesions occurred anal/perianal and/or genital/perigenital. In four cases single lesions occurred in other regions of the body. Fever occurred in three patients, of which two had bacterial superinfection. In four cases inguinal lymphadenitis was described, in one case jugular lymphadenitis occurred, whereas six patients did not present with lymphadenitis. All patients received symptomatic therapy. Two patients received antibiotics due to suspected bacterial superinfection. [1] on Bictegravir, Emtricitabin and Tenofovir alafenamide, viremia 22 HIV copies/ml, CD4+ 360/µl. [2] on Dolutegravir and Lamivudin, viremia not detectable, CD4+ 279/µl.

| ID | Sex | Transmission | History of antiretroviral vaccination | Age | Comorbidities | Concomitants | HIV status | Distribution of lesions | Fever | Lymphadenitis | Systemic symptoms | Bacterial infection | Days post symptom onset of first consultation | Hospitalization | Treatment |
|----|-----|-------------|--------------------------------------|-----|--------------|-------------|------------|------------------------|-------|--------------|-------------------|-------------------|-------------------|---------------|----------|
| 01 | M   | MSM         | HIV positive, CCR5, positive, OUD  | 30–40 | HIV positive | positive, OUD | positive, CCR5 | 10 | genital, perigangl., lymph nodes, lesion | yes | none | lymphadenopathy, fever | none | 4 | yes | local therapy, pain medication |
| 04 | M   | MSM         | HIV positive | 30–40 | HIV positive | positive, CCR5, positive, OUD | positive, CCR5 | > 39 | genital, anal, perigangl.| none | inguinal lymphadenitis, fever | none | 10 | yes | local therapy, analgesic therapy |
| 05 | M   | MSM         | HIV negative | 30–40 | HIV negative | negative | no | anal, perigangl., lesion | no | none | none | none | 17 | no | local therapy |
| 06 | M   | MSM         | HIV negative | 30–40 | HIV negative | negative | 10 | perigangl., lesion | no | inguinal lymphadenitis | none | 3 | no | local therapy |
| 11 | M   | MSM         | HIV negative | 30–40 | HIV negative | negative | 8 | anal, perigangl., lesion | yes | none | perigangl. swelling and pain, fever | yes | 7 | yes | analgesic, local therapy |
| 02 | M   | MSM         | HIV negative | 30–30 | HIV negative | negative | 8 | genital, perigangl.| no | inguinal lymphadenitis | none | 11 | yes | local therapy |
| 03 | M   | MSM         | HIV negative | 22–29 | HIV negative | negative | 7 | genital, perigangl.| no | none | none | none | 5 | no | local therapy |
| 08 | M   | MSM         | HIV negative | 40–50 | HIV negative | negative | 8 | upper arm, lesion, lesion | no | no | musculoskeletal pain, fever | no | 5 | no | local therapy |
| 06 | M   | MSM         | HIV negative | 30–30 | HIV negative | negative | 3 | genital, perigangl.| yes | inguinal lymphadenitis, fever | yes | 7 | yes | analgesic, local therapy |
| 07 | M   | MSM         | HIV negative | 30–40 | HIV negative | negative | 2 | genital, perigangl.| yes | inguinal lymphadenitis | none | no | no | local therapy |

Abbreviations

MSM male having sex with male
ART anti-retroviral therapy
IQR inter-quartile range
in only two. Lymphadenopathy occurred in five of ten patients and fever only in three, two of which also had developed bacterial superinfection of skin lesions. Patient characteristics are compiled in detail in Table 1. Moderately elevated C-reactive protein (CrP)-levels were observed in eight of ten cases. A detailed overview of laboratory parameters is available in supplementary Table 1.

Of note, two of ten patients were HIV-positive (patients 1 and 4, both CDC Stadium A2, under ART) and presented with considerably more lesions (>30) than HIV-negative patients (patients 3, 4, 5–10), while also exhibiting the highest viral loads in blood (Table 1).

Of note, seroconversion was successfully demonstrated for patient 4 through immunofluorescence test (IFT) by day 34 after symptom onset (supplementary figure 2).

3.2. Initial testing results

Initial testing was performed between day three and day 17 after onset of symptoms, but median times were markedly lower in outpatients (7, [IQR: 5–9 days]) than in hospitalized patients (9, [IQR: 7–10 days]) (see supplementary figure 3A). All initial lesion swabs were positive for monkeypox virus-DNA, with the vast majority at, or above 10⁶ cp/ml. In contrast, oropharyngeal swabs rarely exceeded 10⁶ cp/ml, frequently fell below 10³ cp/ml and some returned negative in both outpatients and hospitalized patients. (See supplementary figure 3B).

3.3. Viral DNA-load dynamics over time in lesion swabs, oropharyngeal swabs and blood

Monkeypox virus-DNA levels were observed in hospitalized patients.
throughout their stay and in follow-up visits (65 samples in total from five different patients). Viral DNA-loads in lesion swabs were consistently at or above $10^6$ cp/ml during the first two weeks after symptom onset (1st week: median 3.31E+07 cp/ml; 2nd week: median 3.04E+06 cp/ml) and only declined below $10^3$ cp/ml during the third week (median: 8.55E+03 cp/ml); however, all lesion swabs were positive for monkeypox virus-DNA over the entire time course (Fig. 1a).

Oropharyngeal swabs were negative in two patients and exhibited consistently lower viral-DNA loads (largely below $10^6$) and a continuous downwards trend during the entire observation period in the others. (1st week: median 8.44E+04 cp/ml; 2nd week: median 4.04E+03 cp/ml; 3rd week: median 0 cp/ml) (see Fig. 1b).

Similarly, blood samples were positive for monkeypox virus-DNA in only four of five patients, with viral DNA-loads at, or below $10^3$ cp/ml and continuously declined throughout the observation period (1st week: median 5.85E+02 cp/ml; 2nd week: median 7.80E+00 cp/ml; 3rd week: single sample, 2.37E+01 cp/ml) (Fig. 1c).

3.4. DNA loads in lesion swab samples remain high despite evolving morphology of pustulae

Viral DNA-loads from different specimen types were compiled for
each patient (Fig. 2a and supplementary figure 4). Photo documentation of cutaneous lesions was performed for patient 1 throughout management and images of an exemplary lesion are depicted in Fig. 2b. Despite dramatic morphological changes throughout the first two weeks, lesion swab samples received during this time were plateauing at very high levels (over 10^7 cp/ml), while DNA-loads in all other materials were gradually declining.

3.5. Whole-genome sequencing of the first monkeypox cases in the series

The monkeypox virus genome sequences derived from lesions of patients 1, 2, 4, 5 and 6 was confirmed by shotgun metagenome sequencing. Moreover, phylogenetic analysis of the deduced consensus sequence, derived from patient 1, with previously reported monkeypox sequences confirmed the affiliation of all sequenced cases to the ongoing multi-country monkeypox outbreak. (Fig. 3a, 3b and supplementary material 1)

3.6. Infectivity in cell-culture experiments

Viral culture was attempted in first available samples of two patients (patient 1: lesion swab, oropharyngeal swab and blood; patient 2: lesion swab and oropharyngeal swab; undiluted inoculum, see supplementary material 1). In both cases, infectious virus was successfully isolated from lesion swabs (viral DNA inoculum/well: 5.33mio copies and 4.99mio copies), but not from oropharyngeal swabs or blood (viral DNA inoculum/well: 16,872 copies and 211 copies).

4. Discussion and conclusion

This study represents one of the first clinical case series from the ongoing monkeypox virus outbreak including serial viral DNA-load measurements in different specimen types throughout the course of disease. Different from previous monkeypox clusters outside endemic regions in Africa, most patients presented with rather mild absent systemic symptoms, which is consistent with recent reports from the 2022 outbreak ([7, 8, 18]).

Longitudinal observation of viral DNA-load kinetics demonstrated the reliability of cutaneous lesion swab samples for monkeypox virus detection, which are considered the gold standard for diagnostics ([10, 19]). In this study, lesion swabs never returned negative in infected patients, even at later stages of disease; however, very high concentrations of viral DNA and the ability to infect cell culture, especially during the first two weeks after symptom onset, may have implications for risk of contamination and personnel safety. It should be noted that viral DNA-copies are not indicative of the amount of infectious viral particles.

Other clinical material such as blood and oropharyngeal swabs were recently reported to contain detectable monkeypox virus-DNA [11]; however, throat swab samples are known to be unreliable and difficult to standardize, e.g. in SARS-CoV-2 diagnostics [20]. Blood and oropharyngeal swabs were consistently PCR-negative in 1/5 and 2/5 patients of our cohort respectively, thus making them unreliable standalone specimen types for primary diagnosis. However, their potential value for pre-/asymptomatic cases remains to be established [9]. Interestingly, the highest levels of viral-DNA in blood were detected in two HIV-positive patients under ART and coincided with substantially increased numbers of pustulae. Therefore, viremia as a parameter for monitoring and risk assessment, as well as potentially increased risk of severe disease in HIV patients despite adequate therapy, warrants further investigation.

CRediT authorship contribution statement

Dominik Nörz: Methodology, Investigation, Writing – original draft, Writing – review & editing. Thomas Theo Brehm: Investigation, Resources, Writing – original draft, Writing – review & editing. Hui Ting Tang: Investigation, Resources, Writing – review & editing. Ilka Grewe: Investigation, Resources, Writing – original draft, Writing – review & editing. Lennart Hermannsu: Investigation, Resources, Writing – review & editing. Hanna Matthews: Investigation, Resources, Writing – review & editing. Julia Pestel: Investigation, Resources, Writing – review & editing. Olaf Degen: Resources, Writing – review & editing. Thomas Günther: Methodology, Investigation, Resources, Writing – review & editing. Adam Grundhoff: Methodology, Resources, Writing – review & editing. Nicole Fischer: Methodology, Resources, Writing – review & editing. Marylyn M. Addo: Resources, Writing – review & editing. Sabine Jordan: Resources, Writing – review & editing. Sandra Hertling: Resources, Writing – review & editing. Stefan Unger: Resources, Writing – review & editing. Guido Schäfer: Resources, Writing – review & editing. Knud Scheue: Resources, Writing – review & editing. Christian Hoffmann: Resources, Writing – review & editing. Martin Aepfelbacher: Supervision, Writing – review & editing. Susanne Pfefferle: Methodology, Investigation, Resources, Writing original draft, Writing – review & editing. Julian Schulze zur Wiesch: Conceptualization, Resources, Writing – review & editing. Supervision. Stefan Schmiedel: Conceptualization, Resources, Writing – review & editing. Supervision. Marc Lütgethetmann: Conceptualization, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

ML and DN received speaker honoraria and related travel expenses from Roche Diagnostics. All other authors declare no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2022.105254.

References

[1] ECDC, Monkeypox multi-country outbreak, first update – 8 July 2022, ECDC Website (2022).
[2] M Kozlov, Monkeypox goes global: why scientists are on alert, Nature 606 (2022) 15–16.
[3] A. Adelaja, T Inglesby, A novel international monkeypox outbreak, Ann. Intern. Med. (2022), https://doi.org/10.7326/M22-1581 doi.
[4] A. Antinori, V. Mazzotta, S. Vita, F. Curletti, D. Tacconi, A. D’Abramo, S. Giclan1, D. Lapa, S. Pitral1, V. Puro, M. Rivano Capparuccia, E. Giombini, C.E. M. Gruber, A.R. Garbuglia, A. Marani, F. Vairo, E. Girardi, F. Vaia, E. Nicastri, Group tIM, Epidemiological, clinical and virological characteristics of four cases of monkeypox support transmission through sexual contact, Italy, May 2022, Eurosurveillance 27 (2022), 2200421.
[5] R. Vivancos, C. Anderson, P. Blomquist, S. Balasegaram, A. Bell, L. Bishop, C. Brown, Y. Chow, O. Edeghere, I. Florence, S. Logan, P. Manley, W. Crowe, A. McAuley, A.G. Shankar, B. Mora-Peris, K. Farrahnamathan, M. Prochazka, C. Ryan, D. Simon, R. Vipoud, C. Byers, N.A. Watkins, team UMM, W. Welfare, E. Whittaker, C. Dewsnap, A. Wilson, Y. Young, M. Chand, S. Riley, S. Hopkins, Community transmission of monkeypox in the United Kingdom, April to May 2022, Eurosurveillance 27 (2022), 2200422.
[6] M. Perez Duque, S. Ribeiro, J.V. Martins, P. Casaca, P.P. Leite, M. Tavares, K. Mansinho, L.M. Duque, C. Fernandes, R. Cordeiro, M.J. Borrego, A. Pelerito, I. L. de Carvalho, S. Núñez, V. Manageiro, C. Minetti, J. Machado, J.M. Hausig, R. Crozi, G. Spiteri, A.S. Gual, D. Mendes, T. Souto, S. Pocinho, T. Fernandes, A. Firme, P. Vasconcelos, G. Freitas, Ongoing monkeypox virus outbreak, Portugal, 29 April to 23 May 2022, Eurosurveillance 27 (2022), 2200424.
[7] K.D. Reed, J.W. Melski, M.B. Graham, R.L. Regney, M.J. Soir, M.V. Wegner, J. J. Kaminski, E.J. Stratman, Y. Li, J.A. Fairley, G.R. Swain, V.A. Olson, E. Kazmierczak, E.J. Stratman, Y. Li, J.A. Fairley, G.R. Swain, V.A. Olson, E. Whittaker, C. DeWsnap, A. Wilson, Y. Young, M. Chand, S. Riley, S. Hopkins, Group tIM, Epidemiological, clinical and virological characteristics of four cases of monkeypox support transmission through sexual contact, Italy, May 2022, Eurosurveillance 27 (2022), 2200421.
[8] I. De Baetselier, C. Van Dijck, C. Kenyon, J. Coppens, D. Van den Bossche, H. Smet, L. Lisensborges, F. Vanroye, T. De Block, A. Rezende, E. Florence, K. Vercauteren, M. Van Esbroeck, Asymptomatic monkeypox virus infections among male sexual health clinic attendees in Belgium, medRXiv (2022), https://doi.org/10.1101/2022.07.04.22277226.2022.07.04.22277226 doi.
[9] World Health O, Laboratory testing for the monkeypox virus: interim guidance, 23 May 2022, World Health Organization, Geneva (2022).
[11] H. Adler, S. Gould, P. Hine, L.B. Snell, W. Wong, C.F. Houlihan, J.C. Osborne, T. Rampling, M.B.J. Beadsworth, C.J.A. Duncan, J. Dunning, T.E. Fletcher, B. Hunter, M. Jacobs, S.H. Khoo, W. Newsholme, D. Porter, R.J. Porter, L. Ratcliffe, M.G. Semple, A.J. Tunbridge, T. Wingfield, N.M. Price, M. Abouyannis, A. Al-Balushi, S. Aston, R. Ball, N.J. Beeching, T.J. Blanchard, F. Carlin, G. Davies, A. Gillespie, S.R. Hicks, M.-C. Hoyle, C. Ilozue, L. Mair, S. Marshall, A. Neary, E. Nsutebu, S. Parker, H. Ryan, L. Turtle, C. Smith, J. van Aartsen, N.F. Walker, S. Woolley, A. Chawla, I. Hart, A. Smielewska, et al., Clinical features and management of human monkeypox: a retrospective observational study in the UK, The Lancet Infect. Dis. 22 (8) (2022) 1153–1162, https://doi.org/10.1016/S1473-3099(22)00228-6.

[12] J. Isidro, V. Borges, M. Pinto, D. Sobral, J.D. Santos, A. Nunes, V. Muxio, R. Ferreira, D. Santos, S. Duarte, L. Vieira, M.J. Borrego, S. Núncio, L.L. de Carvalho, A. Pelerito, R. Cordeiro, J.P. Gomes, Phylogenomic characterization and signs of microevolution in the 2022 multi-country outbreak of monkeypox virus, Nat. Med. (2022), https://doi.org/10.1038/s41591-022-01907-y.

[13] D. Norz, H.T. Tang, P. Emmerich, K. Giersch, N. Fischer, M.M. Addo, M. Aepfelbacher, S. Pfefferle, M. Lütgehetmann, Rapid adaptation of established high-throughput molecular testing infrastructure for detection of monkeypoxvirus, Emerg Infect Dis. (2022), https://doi.org/10.3201/eid2809.220917 doi.

[14] D. Norz, S. Pfefferle, T.T. Brehm, G. Franke, I. Grewe, B. Knobling, M. Aepfelbacher, S. Pfefferle, M. Lütgehetmann, Rapid adaptation of established high-throughput molecular testing infrastructure for detection of monkeypoxvirus, Emerg Infect Dis. (2022), https://doi.org/10.3201/eid2809.220917 doi.

[15] T. Gunther, L. Haas, M. Alawi, P. Wohlslein, J. Marks, A. Grundhoff, P. Becher, N. Fischer, Recovery of the first full-length genome sequence of a parapoxvirus directly from a clinical sample, Sci. Rep. 7 (2017) 5734.

[16] Y. Li, V.A. Olson, T. Laue, M.T. Laker, I.K. Damon, Detection of monkeypox virus with real-time PCR assays, J. Clin. Virol. 36 (2006) 194–203.

[17] S.N. Shchelkunov, D.N. Shcherbakov, R.A. Makayutov, E.V. Gavrilova, Species-specific identification of variola, monkeypox, cowpox, and vaccinia viruses by multiplex real-time PCR assay, J. Virol. Methods 175 (2011) 163–169.

[18] F.S. Minhaj, Y.P. Ogale, F. Whitehill, J. Schultz, M. Foote, W. Davidson, C. M. Hughes, K. Wilkins, L. Bachl, J. Charlet, M.A.P. Donnelly, R. Mendoza, B.L. Downes, M. Roskonk, M. Barnes, G.R. Gallagher, N. Bungo, V. Ruiz, N.T. T. Kyaw, A. Feldpausch, A. Valderrama, F. Alvarado-Ramy, C.H. Dowell, C. C. Chow, V. Li, L. Quilter, J. Brooks, D.C. Daskalakis, R.P. McClung, B.W. Petersen, I. Damon, C. Hutson, J. McQuiston, A.K. Rao, E. Belay, A.M. McCollum, Monkeypox Outbreak - Nine States, May 2022. MMWR Morb Mortal Wkly Rep. 71 (2022) 764–769.

[19] D. Li, K. Wilkins, A.M. McCollum, L. Ouaidebe, J. Kahambu, B. Ngute, T. Likafi, M. P. Ballí, R.S. Lushima, J. Malekani, I.K. Damon, M.C.L. Vickery, E. Pakota, F. Nakawa, S. Karhemere, J. J.M. Tamfum, E.W. Okitolonda, Y. Li, M.G. Reynolds, Evaluation of the GeneXpert for human monkeypox diagnosis, The Am. Soc. Tropical Med. Hygiene 96 (2017) 405–410.

[20] N.N.Y. Tsang, H.C. So, K.Y. Ng, B.J. Gowing, G.M. Leung, D.K.M Ip, Diagnostic performance of different sampling approaches for SARS-CoV-2 RT-PCR testing: a systematic review and meta-analysis, The Lancet Infect. Dis. 21 (2021) 1233–1245.