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VIROLOGY

Monkeypox Outbreak 2022: Clinical and Virological Features of 30 Patients at the Sexually Transmitted Diseases Centre of Sant' Orsola Hospital, Bologna, Northeastern Italy

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ABSTRACT Monkeypox infection is a zoonosis first described in humans in 1970 in Congo. While previously manifesting in small, confined outbreaks, the disease is rapidly spreading globally. The aim of this study was to investigate microbiological samples (skin, rectal, and oropharyngeal swab samples and plasma and urine samples) that can help in adequate diagnostic, therapeutic, and prognostic management. We present 30 laboratory-confirmed monkeypox patients with peculiar clinical and virological features admitted to the Sexually Transmitted Diseases Centre of Sant' Orsola Hospital, University of Bologna, in the period between 20 June and 10 August 2022. Demographic, anamnestic, and clinical data were obtained, and microbiological samples were collected and analyzed by real-time PCR to detect the presence of monkeypox virus (MPXV) DNA. All monkeypox patients were adult men who have sex with men (MSM) (mean age, 37.5 years). Nonskin samples were collected from 29 patients during the acute phase of the infection. The detection rates of MPXV DNA in plasma, urine, and oropharyngeal swab samples (82.3%, 64.7%, and 75.0%, respectively) were highest in samples collected 4 to 6 days after symptom onset. The presence of MPXV in plasma and urine samples was analyzed 11 to 38 days after symptom onset to monitor viral shedding duration. Interestingly, MPXV DNA was detected in a urine sample collected on day 21 in one patient. Prolonged positivity in urine after the clinical recovery suggests a potential source of infection by contamination of wastewater and sewage and transmission to possible animal reservoirs and highlights the need for further investigations on nonskin samples to extend and more adequately standardize the patient isolation period.

KEYWORDS monkeypox, public health, urine samples

Monkeypox virus (MPXV), first isolated in captive-bred cynomolgus macaques in a laboratory in Denmark in 1958, is an enveloped double-stranded DNA virus belonging to the *Orthopoxvirus* genus of the *Poxviridae* family. MPXV has animal reservoirs; naturally infected species include various rodents (squirrels, rats, mice, and porcupines) and primates (1). The infection was first described in humans in 1970 in the Congo Basin and later occurred in outbreaks in rural regions of Central and Western Africa countries, emerging as the most important orthopoxvirus for public health after the eradication of smallpox in 1980. In 2003, the first outbreak outside Africa was reported in the United States, followed by other sporadic cases described in the United Kingdom, Israel, and Singapore (2–4). Since May 2022, multiple cases of monkeypox have been identified in several nonendemic countries, 917 of which were reported in Italy (source, ECDC), raising serious concerns for public health authorities (5).

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As far as human-to-human transmission is concerned, the mechanisms reported in the literature so far are close physical contact with skin lesions of an infected person, respiratory droplets, body fluids, or contaminated fomites (3). Recently, MPXV DNA was found in seminal fluid (6). Although not classically described as a sexually transmissible infection (STI), monkeypox can be transmitted through intimate contact. The current outbreak in nonendemic countries is showing peculiar clinical and behavioral features in almost all the monkeypox cases, suggesting rapid growth and predominant spread among men who have sex with men (MSM) (7). In endemic African countries, there are limited observations of possible sexually associated outbreaks, but these cannot be completely excluded.

Monkeypox is responsible for a wide range of systemic symptoms similar to those seen in the past in smallpox; it is characterized by a rash with asynchronous macular-papular-pustular-vesicular lesions but is usually of mild severity and self-resolving (8).

MATERIALS AND METHODS

We present a case series of monkeypox patients diagnosed at the Sexually Transmitted Diseases Centre of IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy, in a 2-month period (20 June to 10 August 2022).

The demographic and anamnestic data collected were age, biological sex, education level, sexual behavior, previous STIs, HIV infection, preexposure prophylaxis (PrEP) for HIV, history of smallpox vaccine, and potential sexual exposure to STDs or MPXV. Data on the number, presentation, and location of skin lesions, systemic symptoms, incubation period, and concomitant STDs were also obtained. Digital epiluminescence microscopy of lesions was performed for all patients that came to our attention.

Clinical samples from patients with suspected MPXV infection were collected and submitted for diagnosis at the Regional Reference Centre for Microbiological Emergencies, Microbiology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy, in accordance with the Regional Health Authorities indications. Skin lesion swab samples, plasma and urine samples, and oropharyngeal and rectal swab samples were analyzed by real-time PCR to detect the presence of MPXV DNA.

Skin biopsies were performed with a 4-mm punch, and the histopathological preparation was stained with hematoxylin-eosin and read by expert dermopathologists of the laboratory of Dermatopathology, Dermatology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy.

The collected data were analyzed and grouped into tables to study the epidemiology, clinical manifestations, and virological parameters of MPXV infection.

The patients described in this manuscript have given written informed consent to the publication of their case details. Patient consents were obtained from the patients.

RESULTS

The patients' demographic data and their anamnestic, clinical, and virological features are detailed in Table 1.

In the period between 20 June and 10 August 2022, 30 patients with MPXV infection, laboratory confirmed, came to our attention.

All monkeypox patients were Caucasian adult men (mean age of 37.5 years), all of whom identified as men who have sex with men (MSM) (7). Twelve of the 30 (40%) were HIV-infected patients (with undetectable viral load in highly active antiretroviral therapy), 4 (13.3%) of whom were in preexposure prophylaxis (PrEP), 5 (16.7%) had an ongoing sexually transmitted disease (2 with gonococcal pharyngitis, 2 with early latent syphilis, and 1 with herpes simplex virus 2 [HSV-2] infection), and 23 (76.7%) had a previous diagnosis of sexually transmitted disease. Over 20% (7/30, 23.3%) had a history of smallpox vaccination (documented by the vaccination certificate issued by Italian health authorities). A potential sexual exposure to MPXV (anal or oral insertive or receptive sex) from 4 to 15 days before appearance of the symptoms (median incubation period, 9 days) was referred to by all patients. All patients had previous sexual exposure to an individual known to have monkeypox or had risk factors for sexually transmitted diseases, such as multiple sexual partners in the weeks before their monkeypox diagnosis. Travel to endemic regions was not reported by any patient.

The skin lesions in monkeypox infection have a polymorphic presentation, starting as maculopapular lesions and evolving into vesicles and pustules, which eventually crust over. The lesions can be approximately in the same stage of development but sometimes appear at different stages and timelines as a consequence of autoinoculation (9, 10). All patients presented with mucocutaneous erosive vesicular-pustular eruptions located on the genital region (n = 25 patients, 83.3%), trunk (n = 9, 30%), or in the perianal area (n = 8, 26.7%), with severe and polymorphic presentation in 10 (30%) patients (Fig. 1a to h). Six

TABLE 1 Demographic data and STI history of patients confirmed for monkeypox disease from 20 June to 10 August 2022, Bologna, Italy

Clinical and virological features	Value [median (min–max), no. (%), or %][a]	Comment
Age	37.5 (21–65)	
Male sex	30 (100)	
Education level:		
High school graduate	9 (30.0)	
University student	9 (30.0)	
University graduate	6 (20.0)	
Undeclared	6 (20.0)	
Sexual behavior:		
MSM	30 (100.0)	
Others	0 (0.0)	
HIV infection	12 (40.0)	Already known previously
PrEP	4 (13.3)	
Previous STIs	23 (76.7)	Gonococcal urethritis, *n* = 6 (20.0%); gonococcal pharyngitis, *n* = 8 (26.7%); gonococcal proctitis, *n* = 7 (23.3%); chlamydial urethritis, *n* = 2 (6.7%); chlamydial pharyngitis, *n* = 3 (10.0%); chlamydial proctitis, *n* = 10 (33.3%); venereal lymphogranuloma, *n* = 4 (13.3%); syphilis, *n* = 13 (43.3%); genital HSV infection, *n* = 2 (6.7%); HPV infection, *n* = 1 (3.3%)
Smallpox vaccine	7 (23.3)	Administered in infancy
Epidemiological link:		
Contact with monkeypox-positive person	5 (16.7)	
General risk factors for STIs	30 (100.0)	
Days between symptom onset and diagnosis	4.6 (0–11)	
Localization of skin lesions:		
Genitals	25 (83.3)	
Perianal region	8 (26.7)	
Face	6 (20.0)	
Trunk	9 (30.0)	
Abdomen	8 (26.7)	
Upper limbs	4 (13.3)	
Hands	4 (13.3)	
Lower limbs	1 (3.3)	
No. of lesions:		
1–10	3 (10)	
10–50	21 (70)	
>50	6 (20)	
Systemic symptoms:		
Lymphadenopathy	21 (70.0)	
Fever	10 (33.3)	
Asthenia	17 (56.7)	
Myalgia	17 (56.7)	
Back pain	2 (6.7)	
At least one of the above	30 (100.0)	
Concomitant STIs	5 (16.7)	Gonococcal pharyngitis, *n* = 2 (6.7%); early latent syphilis, *n* = 2 (6.7%); HSV-2 infection, *n* = 1 (3.3%)
Biological-matrix positivity at the time of diagnosis:		Not performed in:
Skin lesion swab sample	100.00	
Oropharyngeal swab sample	75.0	*n* = 18
Plasma	82.3	*n* = 2
Urine	64.7	*n* = 2
Rectal swab sample	100.0	*n* = 27

[a]All percentages, unless otherwise specified, were calculated considering all patients (*n* = 30).

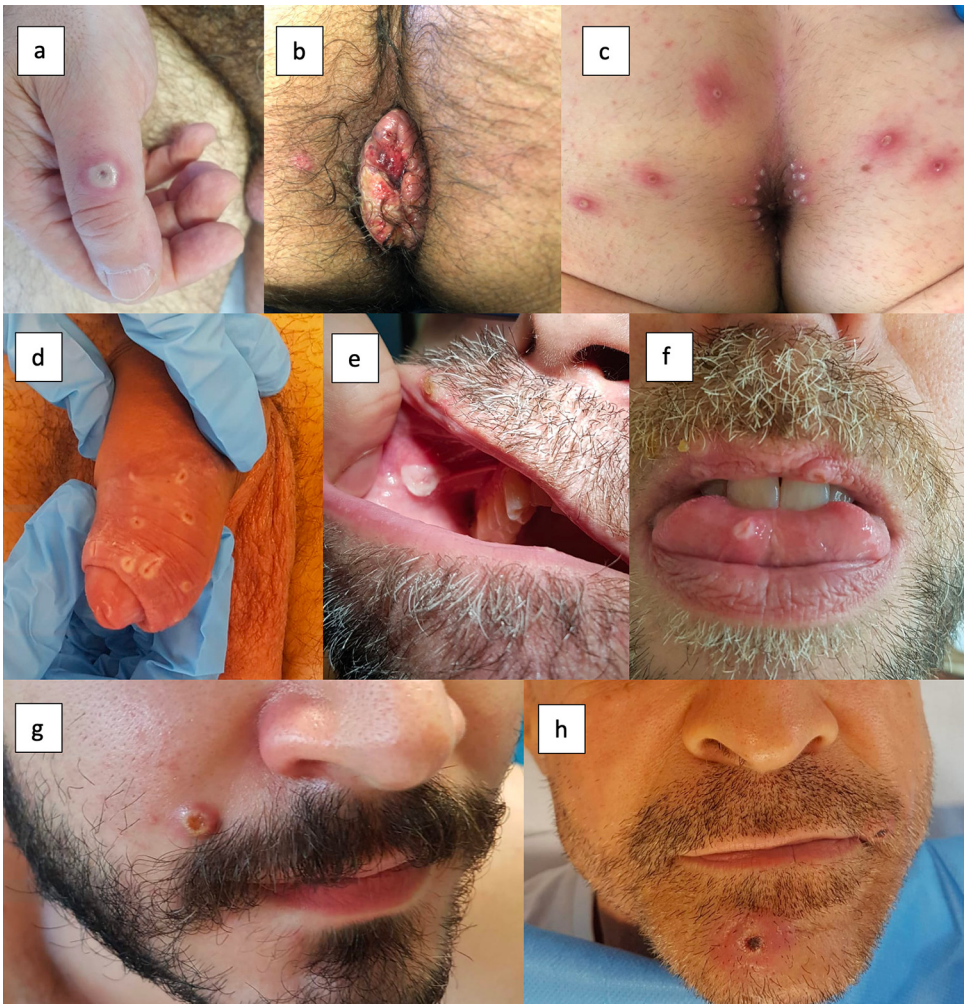

FIG 1 In patients with monkeypox infection, clinical presentation of erythematous vesicles and pustules concentrated on the finger (a), in the perianal region (b and c), and on the foreskin of the penis (d), erosions in the mouth and on the tongue (e and f), and vesicular and crusting lesions on the face (g and h).

patients (20%) had oral erosions. The numbers of lesions were more than 50 in 6 patients (20%), between 10 and 50 in 21 patients (70%), and between 1 and 10 in 3 patients (10%).

The dermoscopy pattern of the typical vesicular-pustular lesion showed a rounded whitish structureless area with a brownish central crust and perilesional erythema. The size and the color varied according to the evolutionary stage of the lesions; the peripheral whitish area progressively disappeared to be replaced by the central crusted red-brown part as the lesion progressed through crusty evolution (Fig. 2a to d).

All (100%) patients reported systemic symptoms, i.e., lymphadenomegaly (n = 21, 70%), myalgia and asthenia (n = 17, 56.7%), and fever (n = 10, 33.3%). Systemic symptoms preceded the onset of skin and mucosal manifestations in 23 patients (70%) and followed them in 7 (30%). Localized symptoms associated with the infection were observed in 27 cases, including rectal pain and proctitis (n = 8, 26.7%), sore throat (n = 6, 20%), tonsillar discomfort (n = 4, 13.3%), and penile edema, swelling, and dysuria (n = 3, 10%). Overall, only 4 (13.3%) patients were admitted to the hospital for the management of symptoms, most commonly proctitis with severe rectal pain or penile edema with difficulty in urination; in the other 26 patients, the symptoms were mild and resolved in 2 to 4 weeks. The patients were advised to isolate themselves at home waiting for the laboratory results and the diagnosis was confirmed in the same day. No deaths were recorded.

The clinical manifestations and their evolution in patients previously vaccinated for smallpox virus were similar to those of unvaccinated patients. Comparing the systemic

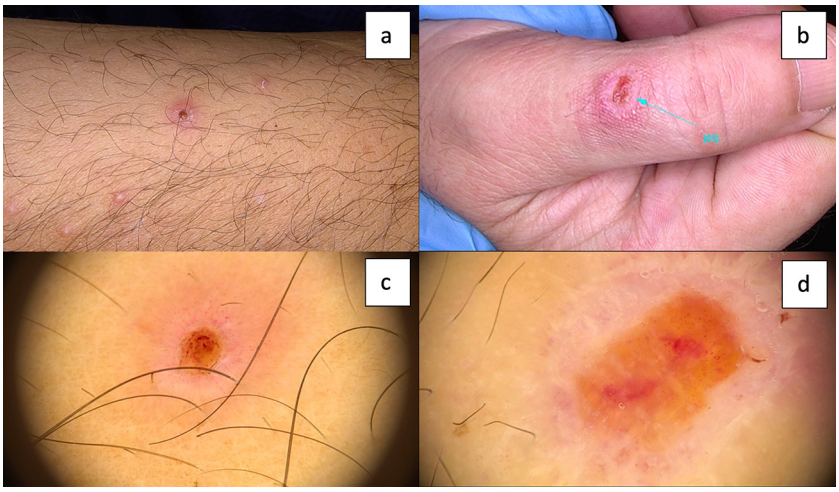

FIG 2 Dermoscopy shows vesicular-pustular lesions comprising a rounded whitish structureless area with a brownish central crust and perilesional erythema. Vesicular-pustular lesions with central umbilication (a, b). Dermoscopy shows a rounded whitish structureless area with a brownish central crust and perilesional erythema (c, d).

manifestations, none of the patients in this cohort presented fever; on the other hand, lymphadenopathy, asthenia, and myalgia were present in percentages comparable to those of unvaccinated patients. An interesting fact is the finding of serological positivity for syphilis and HIV infection in almost all of the patients (6 of 7) previously vaccinated for smallpox virus.

For all patients ($n = 30$), MPXV infection was confirmed by real-time PCR (RealStar orthopoxvirus PCR; Altona Diagnostics GmbH, Germany) of skin lesion swab samples. The median time frame between the onset of symptoms and laboratory confirmation was 5 days (range, 0 to 11 days).

Additionally, nonlesion specimens (plasma and urine samples and oropharyngeal and rectal swab samples) were collected from 29 patients in order to study the presence of MPXV DNA (SYBR green I real-time PCR) (11) during the acute phase of the infection (Table 2). In our cohort, MPXV DNA was very often detected in biological matrices. The data in Fig. 3 summarize the detection rates of MPXV DNA in different specimen types in monkeypox patients at the time of diagnosis. MPXV DNA was detected in plasma samples no later than 7 days after the onset of symptoms. At the time of diagnosis, the detection rates of MPXV DNA in plasma, urine, and oropharyngeal swab samples (82.3%, 64.7%, and 75.0%, respectively) were highest in samples collected 4 to 6 days after the onset of symptoms (Fig. 3). Often, MPXV DNA was detected simultaneously in plasma and urine samples (37.0%). The viral loads in skin lesion swab samples were higher than those in pharyngeal swab, plasma, and urine samples (mean cycle threshold [C_T] values $\pm$ standard deviations, 18.9 $\pm$ 2.7, 31 $\pm$ 4.1, 31.2 $\pm$ 3.1, and 27.3 $\pm$ 4.7, respectively; $P < 0.00001$). (Table 2).

Considering the 8 patients with positive oropharyngeal swab samples, in 6 cases (75%) there were oral lesions, and in all 6 patients with oral lesions, MPXV DNA was detected by PCR in oropharyngeal swab samples (100.0%). For all 6 patients with oral lesions and with MPXV DNA in oropharyngeal swab samples, a history of oral sexual exposure was reported. Finally, rectal swab samples collected from three patients suffering from severe perianal and anal erosions with intense rectal pain had positive results for the presence of MPXV DNA. The data in Fig. 3 summarize the detection rates of MPXV DNA in different specimen types in monkeypox patients.

To monitor the duration of viral shedding, we analyzed the presence of MPXV in plasma and urine samples collected in a longitudinal follow-up in a range of time of 11 to 38 days after the onset of symptoms in eight patients with confirmed monkeypox; oropharyngeal swabs were collected only from two of the eight patients, at 15 and 16 days after symptom onset, and in both cases tested negative for MPXV DNA (Table 2). Interestingly, MPXV DNA

TABLE 2 Virological findings in monkeypox cases diagnosed from June to August 2022, Bologna, Italy

Patient (n = 30)	Time from symptom onset (days)	Result in indicated specimen type (C_T value if positive)[a]				
		Skin lesion swab	Oropharyngeal swab	Plasma	Urine	Rectal swab
1	6	Positive (18)	Positive (39)	Negative	Positive (22)	NA
2	5	Positive (18)	Positive (28)	Positive (34)	Positive (21)	NA
	15	NA	Negative	Negative	Positive (24)	NA
3	4	Positive (15)	NA	Negative	Positive (27)	NA
4	6	Positive (18)	NA	Positive (34)	Positive (19)	NA
5	5	Positive (17)	Negative	Positive (31)	Negative	NA
	16	Positive (29)	Negative	Negative	Negative	NA
6	2	Positive (18)	NA	Positive (31)	Negative	NA
	36	Negative	NA	Negative	Negative	NA
7	4	Positive (21)	NA	Positive (32)	Positive (29)	NA
8	4	Positive (19)	NA	Positive (28)	Positive (32)	NA
9	5	Positive (21)	NA	Positive (29)	Positive (22)	NA
10	5	Positive (18)	NA	Positive (29)	Positive (31)	NA
11	5	Positive (21)	Positive (27)	Positive (28)	Positive (30)	NA
12	5	Positive (19)	Positive (30)	Positive (33)	Negative	NA
13	3	Positive (28)	Negative	Negative	Negative	NA
14	0	Positive (18)	NA	Positive (36)	Negative	NA
15	4	Positive (16)	NA	Positive (32)	Positive (21)	Positive (18)
16	7	Positive (17)	Negative	Negative	Positive (32)	NA
17	7	Positive (18)	NA	Negative	Negative	NA
18	7	Positive (21)	NA	Positive (22)	Negative	NA
19	2	Positive (20)	NA	NA	Negative	NA
	11	Positive (38)	NA	Negative	Negative	NA
20	3	Positive (21)	NA	Positive (34)	Positive (24)	NA
21	5	Positive (20)	NA	NA	NA	NA
	21	Negative	NA	Negative	Positive (28)	NA
22	6	Positive (18)	Negative	Negative	Negative	NA
23	4	Positive (24)	NA	Positive (33)	Negative	NA
	19	NA	NA	Negative	Negative	NA
24	4	Positive (15)	NA	Positive (33)	Negative	NA
	38	Negative	NA	Negative	Negative	NA
25	11	Positive (17)	Positive (31)	Negative	Positive (30)	NA
26	3	Positive (20)	NA	Negative	Negative	NA
27	5	Positive (21)	Positive (28)	Positive (31)	Positive (34)	Positive (33)
28	3	Positive (18)	Positive (35)	Negative	Negative	NA
	20	Positive (29)	NA	Negative	Negative	NA
29	6	Positive (17)	Positive (30)	Positive (31)	Negative	NA
30	3	Positive (16)	NA	Positive (32)	NA	Positive (35)

[a]Positive, detection of MPXV DNA; negative, no detection of MPXV DNA; NA, not available.

was detected in a urine sample collected on day 21 after symptom onset in one patient. The extended positivity in the urine could probably be related to active replication of the virus in the genitourinary tract, which may last longer than in the skin.

After the virological diagnosis, the confirmed cases were reported to the government health authorities taking care of the reporting of patient demographic, epidemiological, and clinical data and close contacts.

In six cases, a skin biopsy was performed. Histological examination showed necrotic keratinocytes, balloon cells, and acanthosis in the epidermis and nodular inflammatory infiltrate in the dermis composed of lymphocytes, neutrophils, and eosinophils (Fig. 4a and b).

DISCUSSION

Our clinical and virological findings confirm the ongoing outbreak of monkeypox infection in a sexually restricted network (men who have sex with men [MSM]) seen in Italy and in many other nonendemic countries. MPXV infection should be suspected in all patients presenting acute onset of a vesicular-papular-pustular eruption in the anogenital region, appearing from 4 to 15 days after anal or oral insertive sex and associated with systemic symptoms (lymphadenopathies, fever, asthenia, myalgia, and back pain). Further research

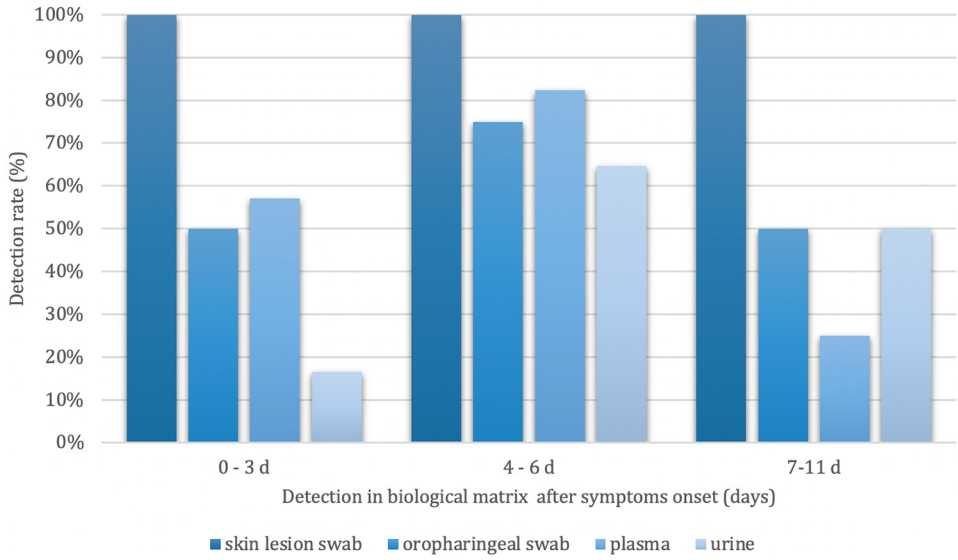

FIG 3 Detection rates of MPXV DNA in different specimen types collected at the time of diagnosis based on days after symptom onset (n = 28).

is needed to clarify the modes of transmission of monkeypox virus, particularly the sexual diffusion, and also the possibility of asymptomatic spread (10).

In agreement with reports by other authors, the majority of the patients had not been vaccinated against smallpox, confirming the role of a waning herd immunity as a major factor in the occurrence of the ongoing outbreak (12). However, over 20% (7/30, 23.3%) were vaccinated, suggesting that the immune coverage is no longer adequate to cross-protect against all orthopoxviruses. The finding that fever is not present in vaccinated patients is likely to be related to the less aggressive innate immune response than in the unvaccinated. The fact that almost all (6 of 7) patients previously vaccinated for smallpox also had serological positivity for syphilis and HIV infection was probably due to the fact that these patients shared the same risk factors for these diseases and, being older, had a higher cumulative risk of acquiring sexually transmitted infections.

Our knowledge about the dynamics of MPXV shedding is still very limited, and reports on the presence and persistence of the virus in different body fluids might help to elucidate mechanisms of virus pathogenesis and transmission. Data on MPXV detection in biological samples have been reported for several cohorts of patients (6, 13–15). In this study, we provide data on nonlesion specimens from 29 patients with monkeypox virus infection. In our cohort of patients, MPXV DNA was frequently detected in plasma, urine, and oropharyngeal swab samples collected during the acute phase of the infection (0 to 11 days after the symptom onset), and the different patterns of viremia and viruria observed might reflect differences in the efficiency of viral clearance by the host immune response. Prolonged shedding of MPXV in urine might be a sign of kidney involvement in viral infection. Interestingly, we report that one patient excreted virus in their urine after clinical recovery (21 days after symptom onset), showing a persistence of PCR positivity in this pathological material during the follow-up. These virological data represent a potential source of infection if the virus excreted with urine is shown to be replication competent. Moreover, this finding may suggest the need to extend the isolation period for convalescents and prompt testing of wastewater and sewage that easily come into contact with synanthropic reservoir species, such as rodents (16).

Skin lesion swab samples showed higher viral loads than respiratory, blood, and urine samples, which combined with the history of sexual exposure and the distribution of lesions suggests that close skin-to-skin contact is probably the dominant transmission route of monkeypox infection during the current 2022 outbreak. As already discussed by Tarín-Vicente et al. (15), different from previous monkeypox cases with prolonged MPXV DNA detection in the upper respiratory tract (17), the recent evidence of low viral loads in respiratory and

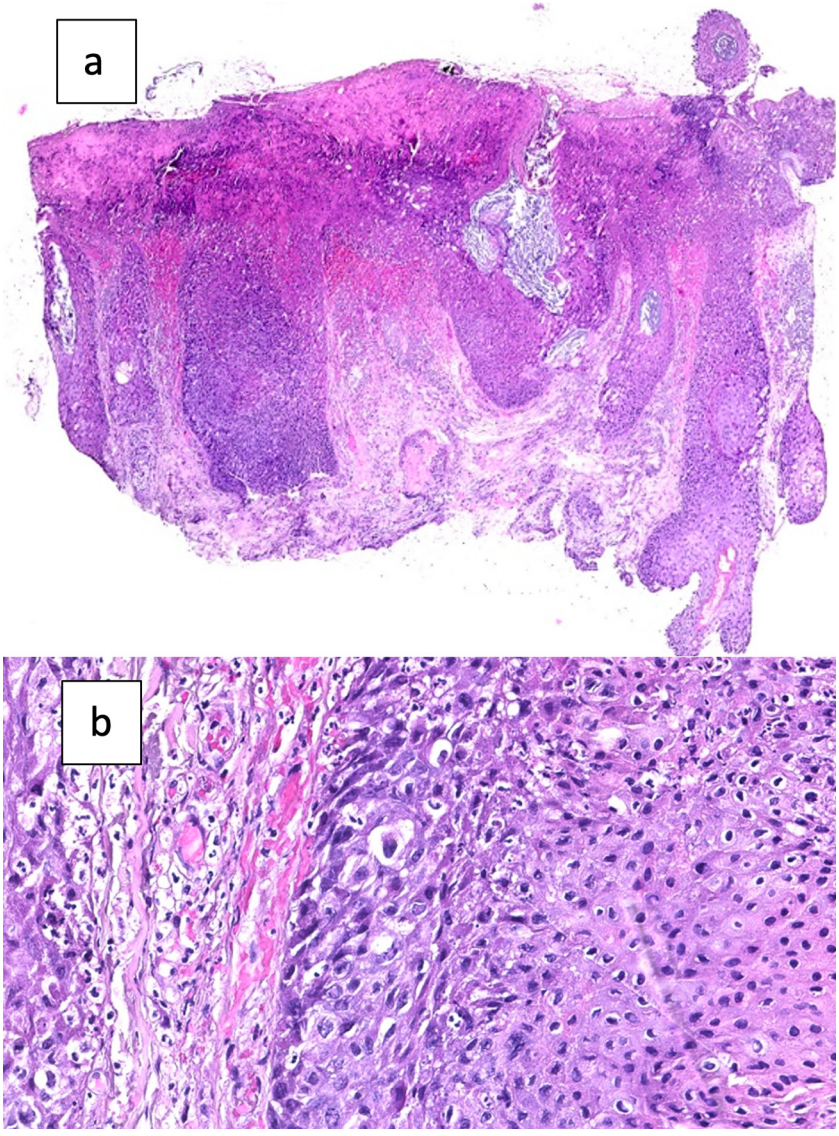

FIG 4 Hematoxylin Eosin staining revealed squamous-crusted ulcerated nodule with dense mixed mostly suppurated infiltate in the dermis (H&E, x 4, a); necrotic keratinocytes and balloon cells in the epidermis (H&E, x 40, b).

blood samples, together with the presence of locally restricted lesions around the point of entry of the virus, may suggest local replication of the virus at the point of entry followed by a low-grade viremia that probably does not support the diffuse dissemination of the infection to the skin and respiratory tract.

Further studies on larger cohorts of patients are needed to assess the frequency, duration, and infectivity of MPXV in different body fluids for a better understanding of viral tropism and the spreading of monkeypox infection.

Although public health guidelines indicate the vesicle as the most representative specimen for the diagnosis of monkeypox, it is important to consider that this virus is epitheliotropic and therefore able to replicate effectively even in nonexplorable mucous membranes, such as the urethral and rectal ones, and consequently, urine and rectal swab samples are particularly important to understand the timing of excretion and thus best define the required patient isolation time after remission of skin symptoms.

The recent experience with the coronavirus disease 2019 (COVID-19) pandemic and its effects on public health, the economy, and society and the growing number of monkeypox

cases detected in a short period underline two aspects of dealing with the monkeypox outbreak.

The first aspect is the need for raising awareness about preventing and recognizing possible clinical manifestations of the disease and encouraging the population to follow prevention guidelines. According to the CDC, it will be crucial to develop appropriate strategies to avoid polarized communication that on the one hand, may pose the risk of discrimination and stigmatization of the most affected community, and on the other hand, may create in heterosexuals the perception of not being at risk of infection.

The second aspect is represented by the importance for clinicians of an accurate and timely diagnosis and of adequate case management with effective containment of confirmed cases, contact tracing, and public hygiene measures aimed at countering the spread of human monkeypox infection on a larger scale.

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Valeria Gaspari and Giada Rossini wrote the paper and coordinated the study, the clinical part and the virological one, respectively; Silvia Robuffo and Luca Rapparini collected the samples and processed clinical data, tables, and photographic images; Alessandra Scagliarini and Alessandra Mistral De Pascali analyzed the pathological samples except skin samples; Bianca Maria Piraccini and Tiziana Lazzarotto supervised the study.

REFERENCES

1. Khodakevich L, Jezek Z, Kinzanzka K. 1986. Isolation of monkeypox virus from wild squirrel infected in nature. Lancet 327:98–99. https://doi.org/10.1016/S0140-6736(86)90748-8.
2. Reed KD, Melski JW, Graham MB, Regnery RL, Sotir MJ, Wegner MV, Kazmierczak JJ, Stratman EJ, Li Y, Fairley JA, Swain GR, Olson VA, Sargent EK, Kehl SC, Frace MA, Kline R, Foldy SL, Davis JP, Damon IK. 2004. The detection of monkeypox in humans in the Western Hemisphere. N Engl J Med 350:342–350. https://doi.org/10.1056/NEJMoa032299.
3. Reynolds MG, Davidson WB, Curns AT, Conover CS, Huhn G, Davis JP, Wegner M, Croft DR, Newman A, Obiesie NN, Hansen GR, Hays PL, Pontones P, Beard B, Teclaw R, Howell JF, Braden Z, Holman RC, Karem KL, Damon IK. 2007. Spectrum of infection and risk factors for human monkeypox, United States, 2003. Emerg Infect Dis 13:1332–1339. https://doi.org/10.3201/eid1309.070175.
4. Alakunle E, Moens U, Nchinda G, Okeke MI. 2020. Monkeypox virus in Nigeria: infection biology, epidemiology, and evolution. Viruses 12:1257. https://doi.org/10.3390/v12111257.
5. Joint ECDC-WHO Regional Office for Europe Monkeypox Surveillance Bulletin [Internet]. monkeypoxreport.ecdc.europa.eu. Accessed 23 November 2022.
6. Lapa D, Carletti F, Mazzotta V, Matusali G, Pinnetti C, Meschi S, Gagliardini R, Colavita F, Mondi A, Minosse C, Scorzolini L, Cicalini S, Maffongelli G, Specchiarello E, Camici M, Bettini A, Baldini F, Francalancia M, Mizzoni K, Garbuglia AR, Nicastri E, Girardi E, Antinori A, Vaia F, Maggi F. 2022. Monkeypox virus isolation from a semen sample collected in the early phase of infection in a patient with prolonged seminal viral shedding. Lancet Infect Dis 22:1267–1269. https://doi.org/10.1016/S1473-3099(22)00513-8.
7. Antinori A, Mazzotta V, Vita S, Carletti F, Tacconi D, Lapini LE, D'Abramo A, Cicalini S, Lapa D, Pittalis S, Puro V, Capparuccia MR, Giombini E, Gruber CEM, Garbuglia AR, Marani A, Vairo E, Girardi F, Vaia F, Nicastri E, INMI Monkeypox Group. 2022. Epidemiological, clinical and virological characteristics of four cases of monkeypox support transmission through sexual contact, Italy, May 2022. Eurosurveillance 27:2200421. https://doi.org/10.2807/1560-7917.ES.2022.27.22.2200421.
8. Bothra A, Maheswari A, Singh M, Pawar M, Jodhani K. 2021. Cutaneous manifestations of viral outbreaks. Australas J Dermatol 62:27–36. https://doi.org/10.1111/ajd.13421.
9. Maronese CA, Beretta A, Avallone G, Boggio FL, Marletta DA, Murgia G, Cusini M, Gori A, Carrera CG, Di Benedetto A, Ramoni S, Marzano AV. 2022. Clinical, dermoscopic and histopathological findings in localized human monkeypox: a case from northern Italy. Br J Dermatol 187:822–823. https://doi.org/10.1111/bjd.21773.
10. Patel A, Bilinska J, Tam JCH, Da Silva Fontoura D, Mason CY, Daunt A, Snell LB, Murphy J, Potter J, Tuudah C, Sundramoorthi R, Abeywickrema M, Pley C, Naidu V, Nebbia G, Aarons E, Botgros A, Douthwaite ST, van Nispen Tot Pannerden C, Winslow H, Brown A, Chilton D, Nori A. 2022. Clinical features and novel presentations of human monkeypox in a central London centre during the 2022 outbreak: descriptive case series. BMJ 378:e072410. https://doi.org/10.1136/bmj-2022-072410.
11. Cheng WE, He X, Jia h, Chen G, Wan C, Zhang J, Jing Z. 2018. Development of a SYBR green I real-time PCR for detection and quantitation of orthopoxvirus by using Ectromelia virus. Mol Cell Probes 38:45–50. https://doi.org/10.1016/j.mcp.2017.12.001.
12. Simões P, Bhagani S. 2022. A viewpoint: the 2022 monkeypox outbreak. J Virus Erad 8:100078. https://doi.org/10.1016/j.jve.2022.100078.
13. Peiró-Mestres A, Fuertes I, Camprubí-Ferrer D, Marcos MA, Vilella A, Navarro M, Rodriguez-Elena L, Riera J, Català A, Martínez MJ, Blanco JL, Hospital Clinic de Barcelona Monkeypox Study Group. 2022. Frequent detection of monkeypox virus DNA in saliva, semen, and other clinical samples from 12 patients, Barcelona, Spain, May to June 2022. Eurosurveillance 27:2200503. https://doi.org/10.2807/1560-7917.ES.2022.27.28.2200503.
14. Noe S, Zange S, Seilmaier M, Antwerpen MH, Fenzl T, Schneider J, Spinner CD, Bugert JJ, Wendtner CM, Wölfel R. 2022. Clinical and virological features of first human monkeypox cases in Germany. Infection 11:1–6. https://doi.org/10.1007/s15010-022-01874-z.
15. Tarín-Vicente EJ, Alemany A, Agud-Dios M, Ubals M, Suñer C, Antón A, Arando M, Arroyo-Andrés J, Calderón-Lozano L, Casañ C, Cabrera JM, Coll P, Descalzo V, Folgueira MD, García-Pérez JN, Gil-Cruz E, González-Rodríguez B, Gutiérrez-Collar C, Hernández-Rodríguez Á, López-Roa P, de Los Ángeles Meléndez M, Montero-Menárguez J, Muñoz-Gallego I, Palencia-Pérez SI, Paredes R, Pérez-Rivilla A, Piñana M, Prat N, Ramirez A, Rivero Á, Rubio-Muñiz CA, Vall M, Acosta-Velásquez KS, Wang A, Galván-Casas C, Marks M, Ortiz-Romero PL, Mitjà O. 2022. Clinical presentation and virological assessment of confirmed human monkeypox virus cases in Spain: a prospective observational cohort study. Lancet 400:661–669. https://doi.org/10.1016/S0140-6736(22)01436-2.
16. Northey H. 27 July 2022. Sewage reveals spread of monkeypox virus. E&E/Greenwire, Washington, DC. https://www.eenews.net/articles/sewage-reveals-spread-of-monkeypox-virus/.
17. Adler H, Gould S, Hine P, Snell LB, Wong W, Houlihan CF, Osborne JC, Rampling T, Beadsworth MBJ, Duncan CJA, Dunning J, Fletcher TE, Hunter ER, Jacobs M, Khoo SH, Newsholme W, Porter D, Porter RJ, Ratcliffe L, Schmid ML, Semple MG, Tunbridge AJ, Wingfield T, Price NM, NHS England High Consequence Infectious Diseases (Airborne) Network. 2022. Clinical features and management of human monkeypox: a retrospective observational study in the UK. Lancet Infect Dis 22:1153–1162. https://doi.org/10.1016/S1473-3099(22)00228-6.