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Clinical presentation and virological assessment of confirmed human monkeypox virus cases in Spain: a prospective observational cohort study

Eloy José Tarín-Vicente, Andrea Alemany, Manuel Agud-Dios, Maria Ubals, Clara Suñer, Andrés Antón, Maider Arando, Jorge Arroyo-Andrés, Lorena Calderón-Lozano, Cristina Casañ, José Miguel Cabrera, Pep Coll, Vicente Descalzo, María Dolores Folgueira, Jorge N García-Pérez, Elena Gil-Cruz, Borja González-Rodríguez, Christian Gutiérrez-Collar, Águeda Hernández-Rodríguez, Paula López-Roa, María de los Ángeles Meléndez, Julia Montero-Menárguez, Irene Muñoz-Gallego, Sara Isabel Palencia-Pérez, Roger Paredes, Alfredo Pérez-Rivilla, María Piñana, Nuria Prat, Aída Ramirez, Ángel Rivero, Carmen Alejandra Rubio-Muñiz, Martí Vall, Kevin Stephen Acosta-Velásquez, An Wang, Cristina Galván-Casas, Michael Marks*, Pablo L Ortiz-Romero*, Oriol Mitjà**

Summary

Background In May, 2022, several European countries reported autochthonous cases of monkeypox, which rapidly spread globally. Early reports suggest atypical presentations. We aimed to investigate clinical and virological characteristics of cases of human monkeypox in Spain.

Methods This multicentre, prospective, observational cohort study was done in three sexual health clinics in Madrid and Barcelona, Spain. We enrolled all consecutive patients with laboratory-confirmed monkeypox from May 11 to June 29, 2022. Participants were offered lesion, anal, and oropharynx swabs for PCR testing. Participant data were collected by means of interviews conducted by dermatologists or specialists in sexually transmitted infections and were recorded using a standard case report form. Outcomes assessed in all participants with a confirmed diagnosis were demographics, smallpox vaccination, HIV status, exposure to someone with monkeypox, travel, mass gathering attendance, risk factors for sexually transmitted infections, sexual behaviour, signs and symptoms on first presentation, virological results at multiple body sites, co-infection with other sexually transmitted pathogens, and clinical outcomes 14 days after the initial presentation. Clinical outcomes were followed up until July 13, 2022.

Findings 181 patients had a confirmed monkeypox diagnosis and were enrolled in the study. 166 (92%) identified as gay men, bisexual men, or other men who have sex with men (MSM) and 15 (8%) identified as heterosexual men or heterosexual women. Median age was 37·0 years (IQR 31·0–42·0). 32 (18%) patients reported previous smallpox vaccination, 72 (40%) were HIV-positive, eight (11%) had a CD4 cell count less than 500 cells per µL, and 31 (17%) were diagnosed with a concurrent sexually transmitted infection. Median incubation was 7·0 days (IQR 5·0–10·0). All participants presented with skin lesions; 141 (78%) participants had lesions in the anogenital region, and 78 (43%) in the oral and perioral region. 70 (39%) participants had complications requiring treatment: 45 (25%) had a proctitis, 19 (10%) had tonsillitis, 15 (8%) had penile oedema, six (3%) an abscess, and eight (4%) had an exanthem. Three (2%) patients required hospital admission. 178 (99%) of 180 swabs from skin lesions collected tested positive, as did 82 (70%) of 117 throat swabs. Viral load was higher in lesion swabs than in pharyngeal specimens (mean cycle threshold value 23 [SD 4] *vs* 32 [6], absolute difference 9 [95% CI 8–10]; p<0·0001). 108 (65%) of 166 MSM reported anal-receptive sex. MSM who engaged in anal-receptive sex presented with proctitis (41 [38%] of 108 *vs* four [7%] of 58, absolute difference 31% [95% CI 19–44]; p<0·0001) and systemic symptoms before the rash (67 [62%] *vs* 16 [28%], absolute difference 34% [28–62]; p<0·0001) more frequently than MSM who did not engage in anal-receptive sex. 18 (95%) of 19 participants with tonsillitis reported practising oral-receptive sex. The median time from onset of lesions to formation of a dry crust was 10 days (IQR 7–13).

Interpretation In our cohort, monkeypox caused genital, perianal, and oral lesions and complications including proctitis and tonsillitis. Because of the variability of presentations, clinicians should have a low threshold for suspicion of monkeypox. Lesion swabs showed the highest viral loads, which, combined with the history of sexual exposure and the distribution of lesions, suggests close contact is probably the dominant transmission route in the current outbreak.

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*Joint senior co-authors

Dermatology Department (E J Tarín-Vicente MD, M Agud-Dios MD, J Arroyo-Andrés MD, L Calderón-Lozano MD, E Gil-Cruz MD, B González-Rodríguez MD, C Gutiérrez-Collar MD, J Montero-Menárguez MD, S I Palencia-Pérez PhD, C A Rubio-Muñiz MD, A Wang MD, Prof P L Ortiz-Romero PhD) **and Microbiology Department** (M D Folgueira PhD, P López-Roa PhD, M de los Ángeles Meléndez PhD, I Muñoz-Gallego PhD, A Pérez-Rivilla PhD), **Hospital Universitario 12 de Octubre, Madrid, Spain; Skin Neglected Diseases and Sexually Transmitted Infections Section** (A Alemany MD, M Ubals MD, C Suñer PhD, M Vall PhD, C Galván-Casas MD, O Mitjà PhD) **and Infectious Diseases Department** (R Paredes PhD, J M Cabrera MD, P Coll MD, Á Rivero MD), **University Hospital Germans Trias i Pujol, Badalona, Spain; Fight Infectious Diseases Foundation, Badalona, Spain** (A Alemany, M Ubals, C Suñer, J M Cabrera, P Coll, Á Rivero, M Vall, C Galván-Casas, O Mitjà, R Paredes); **Department of Dermatology, Hospital Universitario de Móstoles, Madrid, Spain**

Introduction

Autochthonous cases of monkeypox infection were initially confirmed in England from May 6, 2022,[1] and subsequently throughout Europe.[2] In Spain, as in other countries, the current outbreak has mainly affected men who have sex with men (MSM) without any documented

(C Galván-Casas); **Microbiology Department** (A Antón PhD, M Piñana MD) **and Infectious Diseases Department** (M Arando PhD, V Descalzo MD, J N García-Pérez MD), **Hospital Universitari Vall d'Hebron, Barcelona, Spain; Microbiology Department, Clinical Laboratory North Metropolitan Area, University Hospital Germans Trias I Pujol, Badalona, Spain** (C Casañ PhD, Á Hernández-Rodríguez PhD, A Ramirez MD); **BCN Checkpoint, Projecte dels NOMS—Hispanosida, Barcelona, Spain** (J M Cabrera, P Coll, Á Rivero); **Research Institute Hospital i+12, Madrid, Spain** (M D Folgueira, P López-Roa, M de los Ángeles Meléndez, I Muñoz-Gallego, S I Palencia-Pérez, A Pérez-Rivilla, Prof P L Ortiz-Romero); **Department of Medicine, School of Medicine, Complutense University of Madrid, Madrid, Spain** (M D Folgueira, S I Palencia-Pérez, A Pérez-Rivilla, Prof P L Ortiz-Romero); **Department of Genetics and Microbiology, Autonomous University of Barcelona, Barcelona, Spain** (Á Hernández-Rodríguez); **IrsiCaixa AIDS Research Institute, Badalona, Spain** (R Paredes); **Direcció d'Atenció Primària—Metropolitana Nord, Sabadell, Catalonia, Spain** (N Prat MD); **Radiology Department, Hospital Universitario La Paz, Madrid, Spain** (K S Acosta-Velásquez MD); **Clinical Research Department, London School of Hygiene & Tropical Medicine, London, UK** (M Marks PhD); **Hospital for Tropical Diseases, London, UK** (M Marks); **Division of Infection and Immunity, University College London, London, UK** (M Marks); **Universitat de Vic-Universitat Central de Catalunya (UVIC-UCC), Vic, Spain** (O Mitjà); **School of Medicine and Health Sciences, University of Papua New Guinea, Port Moresby, Papua New Guinea** (O Mitjà)

Correspondence to: Dr Oriol Mitjà, Skin Neglected Diseases and Sexually Transmitted Infections Section, University Hospital Germans Trias i Pujol, Badalona, Spain **oriolmitja@hotmail.com**

Research in context

Evidence before this study

We searched PubMed for articles reporting clinical and virological features of the recently reported outbreak of monkeypox virus, which has affected various European countries. Searches using the key term "monkeypox" with no language restrictions among articles published from May 1, 2022, after the first case was reported, up to Aug 1, 2022, retrieved 268 results. Most publications were letters to the editor, perspectives, and case reports of fewer than ten participants. Three articles reported the results of observational studies on the clinical course of human monkeypox infection. One study reported the clinical findings of 54 outpatients attending a sexual health centre in London, UK. Two other studies described new clinical presentations and complications of the disease, including a study in 528 patients with human monkeypox enrolled from 16 countries outside countries where the disease is endemic, and a single-centre study of 197 patients with human monkeypox in central London.

Added value of this study

In our prospective assessment of 181 patients with new diagnoses of monkeypox, we investigated the relationship between sexual behaviour and clinical presentation, virological features, and progression patterns by systematically collecting information on sexual practices, carrying out a detailed clinical examination and follow-up, and testing for viral presence in specimens obtained from skin lesions, throat, and anal mucosa. We confirmed clinical characteristics observed in other observational analyses, and described in more detail several complications, including a proctitis-related syndrome and ulcerative tonsillitis. Additionally, we report how specific types of sexual practices are related to clinical presentation. Compared with some previous studies, recruitment of all consecutively selected patients from three sexual health clinics helps provide some indication of the number and proportion of different types of clinical presentations. Compared with the three studies outside endemic countries, we used a larger sample size to define the incubation period and to estimate the differences in viral load in different mucocutaneous sites. Our study confirms the short incubation period reported previously and provides an estimate of the time to the dry crust phase of lesions. Importantly, the finding of higher viral loads in skin lesions compared with the upper respiratory tract reinforces the likelihood of skin-to-skin contact as the dominant transmission route, whereas respiratory transmission seems to be less important.

Implications of all the available evidence

The evidence available to date suggests that skin-to-skin contact is the dominant transmission route of the monkeypox virus in this outbreak, whereas respiratory transmission is probably less relevant, encouraging the revision of isolation measures in these patients. Additionally, the presence of atypical manifestations, which might be associated with the body site of viral entry, encourages a low threshold for clinical suspicion of monkeypox, particularly in areas with high transmission rates or in individuals who might be at high risk of contagion. The short incubation period suggests that postexposure vaccination strategies are unlikely to be effective.

history of travel to countries where monkeypox is endemic. By June 27, more than 800 cases had been reported in Spain.[3] The pathogen has been identified as monkeypox virus from the west African clade, which is often associated with milder disease than the Congo basin clade. Nevertheless, some outbreak clade mutations have been identified in proteins involved in virus transmission and virulence.[1]

Infections caused by orthopoxviruses can be classified as either systemic or localised (at the site of virus entry).[4] The type of infection depends on the species of orthopoxvirus, the route of entry, and the species and genus of susceptible animals and their immune status. Typically, generalised infections caused by orthopoxviruses manifest as a diffuse rash. By contrast, clinical descriptions of smallpox cases from early medical writings describe a localised rash at the site of virus entry following cutaneous inoculation.[5,6]

Historically, the signs and symptoms of monkeypox in countries in Africa where it is endemic consisted of a characteristic rash of several hundred simultaneous lesions in multiple regions of the body, including the face, arms, legs, and less commonly the palms, soles, or genitalia.[7–13] The rash was normally preceded by prodromal symptoms such as fever, lymphadenopathy, and influenza-like symptoms. Initial evidence from the current outbreak suggests cases are atypical, with the rash in fewer regions of the body, in particular the genital and perianal areas, without spread to other body regions and with a relative mildness or absence of prodromal symptoms.[14]

Detailed information regarding the epidemiology and the clinical features of monkeypox in the 2022 outbreak is scarce. In this study, we aimed to comprehensively evaluate the epidemiology, clinical features, and virological features of patients diagnosed with monkeypox at three large hospitals in Spain.

Methods

Study design and participants

In this multicentre, prospective, observational cohort study we enrolled all consecutive patients diagnosed with monkeypox from May 11 to June 29, 2022, at three hospitals in Spain (Hospital Universitario 12 de Octubre, Madrid; BCN Checkpoint Sexual Health Clinic, University Hospital Germans Trias i Pujol, Barcelona; and Drassanes Sexual Health Clinic, University Hospital Vall d'Hebron, Barcelona). The first is a public general hospital, and the last two are open-access, community-based sexual health

clinics. Together, the three units treat approximately 100 patients with sexually transmitted infections each day. All participants suspected to have monkeypox were offered triple-site (ie, lesion, anal, and oropharynx swabs) monkeypox PCR testing. A confirmed case of monkeypox was defined as a positive result on high throughput sequencing or real-time RT-PCR assay of skin lesion, anal, or oropharynx swab specimens. Only patients with laboratory-confirmed monkeypox were included in the analysis.

The study was approved by the Ethics Committee of the Hospital Germans Trias i Pujol. Oral informed consent was obtained from all participants. Written informed consent for anonymised publication of images was individually sought and obtained from participants.

Procedures

Twelve dermatologists or specialists in sexually transmitted infections interviewed participants for this study. We obtained demographic, epidemiological, clinical presentation, laboratory, and clinical outcome data using a standardised case report form. Data on sexual history, including sexual practices, and the number of sexual partners were also collected. Clinical outcomes were followed up to July 13, 2022. The case definitions were established before the start of data collection. Broadly, case definitions consisted of participants with one or more papular, vesicular, or pustular skin lesion, or signs or symptoms of proctitis. When a new sign or syndrome was identified, the case definition was agreed upon by all recruiting physicians. If any data were missing or clarification was needed, we obtained the information by direct communication with the patient.

Laboratory confirmation of monkeypox was done at the Spanish National Microbiology Centre reference laboratory before June 6, 2022, and subsequently in local certified tertiary care hospitals. Skin lesion, anal, and oropharynx swabs were collected and examined with real-time RT-PCR. Monkeypox virus DNA was detected by LightMix Modular Orthopox Virus assay (TIB MolBiol, Berlin, Germany) on LightCycler 480 Real-Time PCR equipment (Roche Applied Science, Mannheim, Germany) amplifying a 113-base-pair long fragment of the 14 kDa gene specific to orthopoxviruses. A comprehensive sexual health screen was offered to all individuals, including a fourth-generation enzyme immunoassay for HIV serology, syphilis serology (Alinity i Syphilis TP [Abbott, Chicago, IL, USA] and Axis-Shield RPR [Abbott]), and triple-site *Chlamydia trachomatis, Neisseria gonorrhoeae,* and *Mycoplasma genitalium* screening from a pharyngeal swab, a rectal swab, and a first-void urine sample (Allplex STI Essential Assay [Seegene, Seoul, South Korea] or Aptima Combo 2 assay [for *C trachomatis* and *N gonorrhoeae*; Hologic, Marlborough, MA, USA] and Aptima *M genitalium* assay [Hologic]). Additionally, participants presenting with clinical signs of proctitis were screened for *Treponema pallidum* DNA using PCR in rectal swabs (Allplex STI Genital Ulcer assay; Seegene]) and participants with tonsillitis were screened for group A *Streptococcus* (Abbott SD Bioline rapid antigen detection test [Abbott]). All procedures were done as planned, with no deviations from the approved study protocol.

or

Dr Michael Marks, Clinical Research Department, London School of Hygiene & Tropical Medicine, London, WC1E 7HT, UK
michael.marks@lshtm.ac.uk

Outcomes

In all participants, we described demographics, patient-reported historic smallpox vaccination, comorbidities (including HIV status), epidemiological data (ie, incubation period, exposure to someone with monkeypox, travel, mass gathering attendance, and risk factors for sexually transmitted infections), sexual practices, signs and symptoms on first presentation, virological results at multiple body sites (including analysis of cycle threshold values), co-infection with other sexually transmitted pathogens, and clinical outcomes 14 days after the initial presentation to determine progression of disease.

We classified sexual orientation as heterosexual or MSM, including gay and bisexual men. For the purpose of analysis, we differentiated three presumed routes of infection that might be relevant for pathogenesis: anal-receptive sex in MSM, non-anal-receptive sex in MSM, and non-MSM sex. The first and second categories were mutually exclusive, so participants who had receptive anal intercourse were classified in the first group regardless of whether they had engaged in other types of sexual activity. The incubation period was defined as the interval between the potential earliest date of contact with a presumed transmission source (ie, a person with suspected or confirmed monkeypox) and the potential earliest date of symptom onset (ie, influenza-like symptoms or skin rash). To calculate the incubation period, we excluded participants whose timing of exposure was unclear. We defined at least one systemic feature as the presence of any of influenza-like illness, fever, headache, or arthralgia. Skin rash severity was classified as moderate when there were more than 20 lesions, mild when there were three to 20 lesions, and minimal when there were one or two lesions.[5] Acute proctitis was defined as rectal pain and tenesmus or purulent discharge, tonsillitis as sore throat or trouble swallowing and acute enlargement and reddening of the tonsil or tonsils, moderate to severe penile oedema as swelling of the penile glans or foreskin, such that the retracted foreskin cannot be returned to its anatomic position (ie, paraphimosis), and exanthem as a widespread rash of pink-to-red spots on the trunk, arms, and legs.

Statistical analysis

Continuous variables were expressed as medians and IQRs or ranges, as appropriate. Categorical variables were summarised as absolute values and proportions. No imputation was made for missing data. We described clinical features, including the distribution of skin

	Participants (n=181)
Age, years	37·0 (31·0–42·0)
Sex	
Female	6 (3%)
Male	175 (97%)
Ethnicity	
Spanish	79 (44%)
South and central American	82 (45%)
Other	19 (10%)
Missing data	1 (1%)
Sexual orientation	
Gay men, bisexual men, and other men who have sex with men	166 (92%)
Heterosexual men	9 (5%)
Heterosexual women	6 (3%)
History of smallpox vaccination	32 (18%)
HIV-positive	72 (40%)
Possible exposure to monkeypox	
Regular sexual partner with monkeypox	47 (26%)
Household contact with monkeypox	6 (3%)
Attendance at a Pride event	66 (36%)
Recent travel out of Spain	26 (14%)
Sexual risk factors	
Number of sexual partners in past 14 days	2·0 (1·0–5·0)
Number of sexual partners in past 3 months	6·5 (3·0–16·0)
Sexually transmitted infection in past 12 months	99 (55%)
Use of social media apps to identify sexual partners	107 (59%)
Sex outside of Spain in past 3 months	15 (8%)
Sex with a sex worker	8 (4%)
Use of recreational drugs during sex	57 (31%)
Type of sexual practice	
Vaginal-insertive sex	11 (6%)
Vaginal-receptive sex	6 (100%)*
Anal-insertive sex	131 (72%)
Anal-receptive sex	108 (60%)
Oral-insertive sex	160 (88%)
Oral-receptive sex	158 (87%)

Data are median (IQR) or n (%). *Six (100%) of six female participants.

Table 1: **Demographic and epidemiological characteristics of participants**

See Online for appendix

lesions and the incubation period stratified by the presumed route of exposure, and PCR cycle threshold values by pharyngeal or ulcer swab. Analyses were considered descriptive and exploratory. We compared continuous variables using the *t* test and proportions using a χ^2 test. All tests were two-sided with a significance threshold of 0·05. All analyses were performed with R (version 3.6.2).

Role of the funding source

There was no funding source for this study.

Results

181 patients with monkeypox were assessed at the three participating centres during the study period, all of whom consented to take part in the study (99 at Hospital Universitario 12 de Octubre, 67 at BCN Checkpoint, and 15 at Drassanes). The demographic and clinical characteristics of the participants are shown in table 1. 175 (97%) of 181 participants were male and six (3%) were female. The median age of the participants was 37·0 years (IQR 31·0–42·0, range 19·0–58·0). 72 (40%) participants were HIV-positive, 71 (99%) of whom were on antiretroviral therapy, and eight (11%) had a CD4 cell count of less than 500 cells per µL. No individuals were identified without any potential sexual exposures (table 1) and travel to endemic regions was not reported by any participant.

The median incubation period was 7·0 days (IQR 5·0–10·0, range 1·0–19·0). The numbers of participants with systemic features are shown in table 2. All participants presented with skin lesions; 141 (78%) participants had lesions in the anogenital region, and 78 (43%) had lesions in the oral and perioral region (table 2; figure 1). The number of skin lesions was 20 or fewer in 166 (92%) participants. No patients presented with generalised swelling of the lymph nodes as part of the systemic illness, but localised lymphadenopathy in relation to lesion location was observed in 153 (85%) participants. Complications that required medical treatment were described in 70 (39%) participants, most frequently pain relief for proctitis, tonsillitis, and in participants with anal lesions. 41 (91%) of 45 participants with proctitis reported practising receptive anal sex and five (11%) had concurrent chlamydia or gonorrhoea diagnosed from a rectal swab. Of the 19 participants with tonsillitis, all had white ulcerative lesions on the tonsils and a negative group A *Streptococcus* antigen test, and 18 (95%) reported practising oral-receptive sex. Bacterial abscess with culture confirmation were most often around the perianal area and the face (appendix p 9). 15 (8%) participants presented with preputial oedema or gross oedema of the penile glans resulting in paraphimosis. Eight (4%) participants developed a widespread maculopapular exanthem (table 2), five (3%) were diagnosed with a morbilliform drug eruption related to B-lactams, one (1%) had a viral exanthem, one (1%) had an urticarial exanthem, and one (1%) had an erythema multiforme. We did not identify an alternative infectious cause in these patients. We did not notice any difference in clinical features, including the number of lesions, or incubation period between patients who reported being HIV-positive and those who did not, or between patients who reported receiving smallpox vaccination and those who did not (appendix pp 5–8).

Triple-site swabbing was offered to the 114 participants at two of the three sexual health clinics, but the 67 participants at BCN Checkpoint were offered only lesion swab collection. The proportions of skin, throat,

	Participants (n=181)
Incubation period, days*	7·0 (5·0–10·0)
Systemic features	
At least one systemic feature	160 (88%)
Systemic symptoms before the rash onset	87 (48%)
Influenza-like illness	147 (81%)
Fever	131 (72%)
Headache	96 (53%)
Sore throat	66 (36%)
Clinical features of the rash	
Approximate number of lesions	
>20	15 (8%)
3–20	145 (80%)
1–2	21 (12%)
Number of body regions involved	3 (2–4)
Lesion morphology	
Papular lesions	38 (21%)
Vesicular lesions	47 (26%)
Pustular lesions	162 (90%)
Lesion location	
Genital	100 (55%)
Perianal	66 (36%)
Oral ulcer	45 (25%)
Perioral	51 (28%)
Hands and feet	108 (60%)
Trunk and extremities	104 (57%)
Lymphadenopathies	
Any lymphadenopathy	153 (85%)
Lymphadenopathy by region	
Cervical	53 (29%)
Inguinal	110 (61%)
Axillary	2 (1%)
None	28 (15%)
Complications	
Proctitis	45 (25%)
Tonsillitis	19 (10%)
Penile oedema	15 (8%)
Bacterial skin abscess	6 (3%)
Exanthem	8 (4%)
Investigations	
PCR of skin swab positive†	178/180 (99%)
Mean cycle threshold value of positive skin specimens	23 (4)
PCR of throat swab positive†	82/117 (70%)
Mean cycle threshold value of positive throat specimens	32 (6)
PCR of anal swab positive†	43/55 (78%)
Mean cycle threshold value of positive anal specimens	27 (7)

(Table 2 continues in next column)

(Continued from previous column)

	Participants (n=181)
Concurrent sexually transmitted infection	
Any sexually transmitted infection	31 (17%)
HIV	1 (1%)
Chlamydia	10 (6%)
Gonorrhoea	6 (3%)
Herpes simplex virus	2 (1%)
Mycoplasma genitalium	2 (1%)
Syphilis	13 (7%)
Outcomes	
Time to formation of dry crust, days	10·0 (7·0–12·5)
Admitted to hospital	
No	178 (98%)
Clinical management	2 (1%)
Social reasons	1 (1%)

Data are median (IQR), n (%), n/N (%), or mean (SD). *n=144; 37 participants had missing data. †Denominators are smaller than the total number of participants because some participants did not have these PCR tests done.

Table 2: **Clinical characteristics on first presentation and laboratory results**

and anal swabs that were positive are shown in table 2. The mean cycle threshold value of positive lesion swabs was significantly lower (ie, higher viral load) than from positive pharyngeal swabs (23 [SD 4] *vs* 32 [6], absolute difference 9 [95% CI 8–10]; p<0·0001) (figure 2A) and this was true regardless of where the skin lesions were found (data not shown). The mean cycle threshold value of anal swabs was 27 (SD 7). When we excluded participants with oral lesions or tonsillitis that could cause contamination of throat swabs, 38 (63%) of 60 oropharyngeal specimens were positive, with a mean cycle threshold value of 34 (SD 4). Similarly, when we excluded participants with anal lesions or proctitis, 14 (58%) of 24 anal swabs were positive, with a mean cycle threshold value of 30 (SD 7). Neither time from onset of symptoms nor HIV status was associated with different cycle threshold values for samples (appendix p 4). Sequencing of 23 genomes with 100% coverage of specimens collected in Spain indicates that these genomes belong to the west African clade[15,16] and are almost identical to other genomes uploaded from other European countries. A concurrent sexually transmitted infection on this presentation was diagnosed in 31 (17%) of 181 participants, most commonly chlamydia (n=10) and syphilis (n=13).

MSM who engaged in anal-receptive sex presented with proctitis more frequently than MSM who did not engage in anal-receptive sex (41 [38%] of 108 *vs* four [7%] of 58, absolute difference 31% [95% CI 19 to 44]; p<0·0001; figure 2B, table 3). MSM who engaged in anal-receptive sex also presented with systemic symptoms before the rash more frequently than MSM who did not engage in anal-receptive sex (67 [62%] *vs* 16 [28%], absolute difference 34% [28 to 62]; p<0·0001); there was no difference in incubation times between the two groups (median 8·0 days [IQR 5·0–10·0] *vs* 7·0 days [5·0–9·0], absolute difference 1 day [–1·4 to 1·2]; p=0·88; table 3, figure 2C). Among participants with throat PCR available,

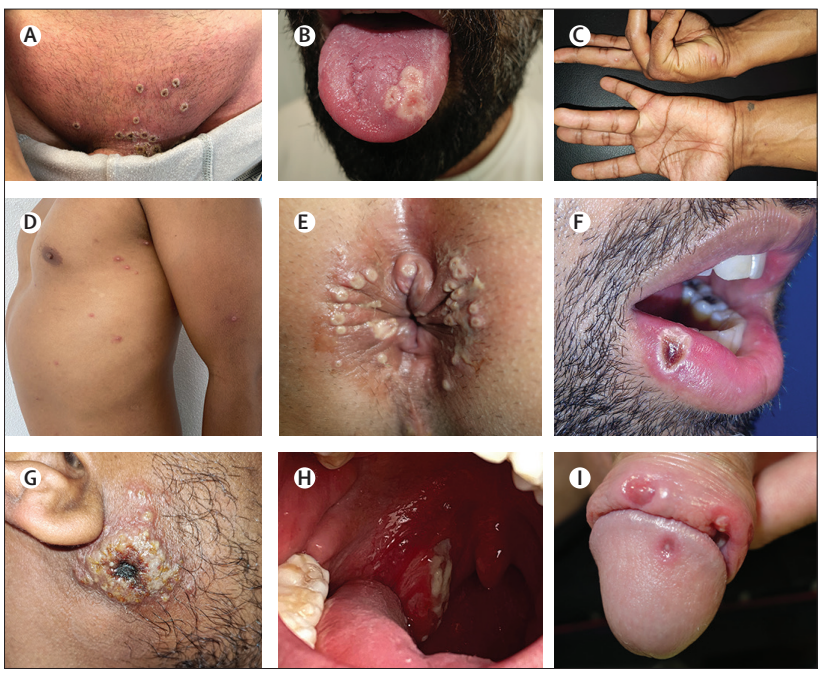

Figure 1: **Clinical presentation of monkeypox**
(A) Pustules in the genital and pubic region, in which the initial umbilication has progressed to necrotic crust with central depression. (B) Three semiconfluent pustular lesions with a depressed centre located on the left side of the tongue dorsum. (C) Pearly acral vesicles embedded in the thick stratum corneum of the palmar skin, shotty on palpation. (D) Scattered papules, pustules, and umbilicated pustules surrounded by an erythematous halo on the lateral aspect of the chest and left arm. (E) Pustules circumferentially distributed on the anal margin and perianal skin. (F) A pustular lesion with a crusted centre on the semimucosa of the lower lip, close to the right oral commissure. (G) Primary inoculation site with a large, crusted lesion on the right cheek. (H) The right palatine tonsil is reddened and enlarged and has a fibrin-covered ulcer. (I) The penile glans and foreskin have lesions of varying sizes and stages of evolution, with oedema surrounding the larger ulcer. Pictures A–C, E–G, and I were taken by EJT-V; pictures D and H were taken by MU.

MSM reporting anal-receptive sex had a higher positivity rate in throat specimens (49 [82%] of 60 *vs* 24 [57%] of 42; p=0·013), presumably reflecting a higher rate of distant dissemination.

Six participants received treatment with topical cidofovir. None received tecovirimat. The median time from the onset of lesions to the formation of a dry crust was 10 days (IQR 7–13, range 2–24) and was broadly similar between people who were HIV-positive (median 11 days [IQR 8–14 days]) and people who were not HIV-positive (median 10 days [7–12]; appendix p 5). The majority of participants were managed as outpatients, with only three (2%) requiring admission to hospital: two (67%) for management of bacterial abscesses and one (33%) for social reasons. There were no deaths.

Discussion

In the early stages of the monkeypox outbreak in 2022, diagnosis and disease control have been difficult because many cases have not followed the patterns of illness described in the medical literature. In concert with recent studies,[1,17–19] we found that most participants presented with a low number of lesions located in one or more of the genital, oral, and anal regions and that systemic symptoms were very common. Almost half of the participants had systemic illness before the rash appeared (prodromal stage) and just over half had systemic illness shortly afterwards (early clinical stage). These symptoms are attributable to the invasive phase of illness, which might sometimes occur after lesions have formed at the site of inoculation. During the invasive phase, the virus might spread to distant areas such as the face, limbs, and trunk and cause lesions at a different stage of progression than the initial local rash. In contrast to previous reports of monkeypox virus infections, no generalised swelling of the lymph nodes was observed, but regional lymphadenopathies were often present in the lymph catchment area of lesions. Nearly all participants 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 12 weeks before their monkeypox diagnosis or use of recreational drugs during sex. The fact that 32 individuals acquired monkeypox despite smallpox vaccination in their childhood is of note and warrants further investigation to better understand the protection provided by vaccination in the context of the current outbreak. Additionally, 40% of individuals were HIV-positive, including eight participants with a CD4 cell count of less than 500 cells per µL. Neither the severity nor the progression of the disease differed between people who were HIV-positive and the rest of the participants. Given the high CD4 counts of participants in this study, we cannot comment on whether more immunosuppressed individuals might develop more severe disease. Due to the sampling strategy, we could not assess whether people who were HIV-positive were more susceptible to monkeypox infection because half of the participants were recruited from a hospital that provides health services to many individuals with HIV.

More than a third of participants presented with complications that required pain-relief medication. The most common complications were proctitis (sometimes extremely painful and in other cases associated with very intense itching), tonsillitis, paraphimosis due to penile oedema, and bacterial abscesses. Participants reporting anal-receptive sex were more likely than others to have early systemic symptoms before developing skin lesions. One explanation is that anal sex might damage the epithelium and enable blood entry, allowing greater viraemia at an early stage when local lesions have not yet developed. An alternative explanation is that these participants did have rectal lesions at the time of initial presentation, but these were missed. A similar phenomenon has been observed in patients with syphilis: MSM are less likely to present with primary syphilis because rectal chancres are often missed.

There are questions about whether monkeypox is sexually transmitted via semen and vaginal secretions. However, the extended definition of sexually transmitted infections such as syphilis and herpes simplex includes

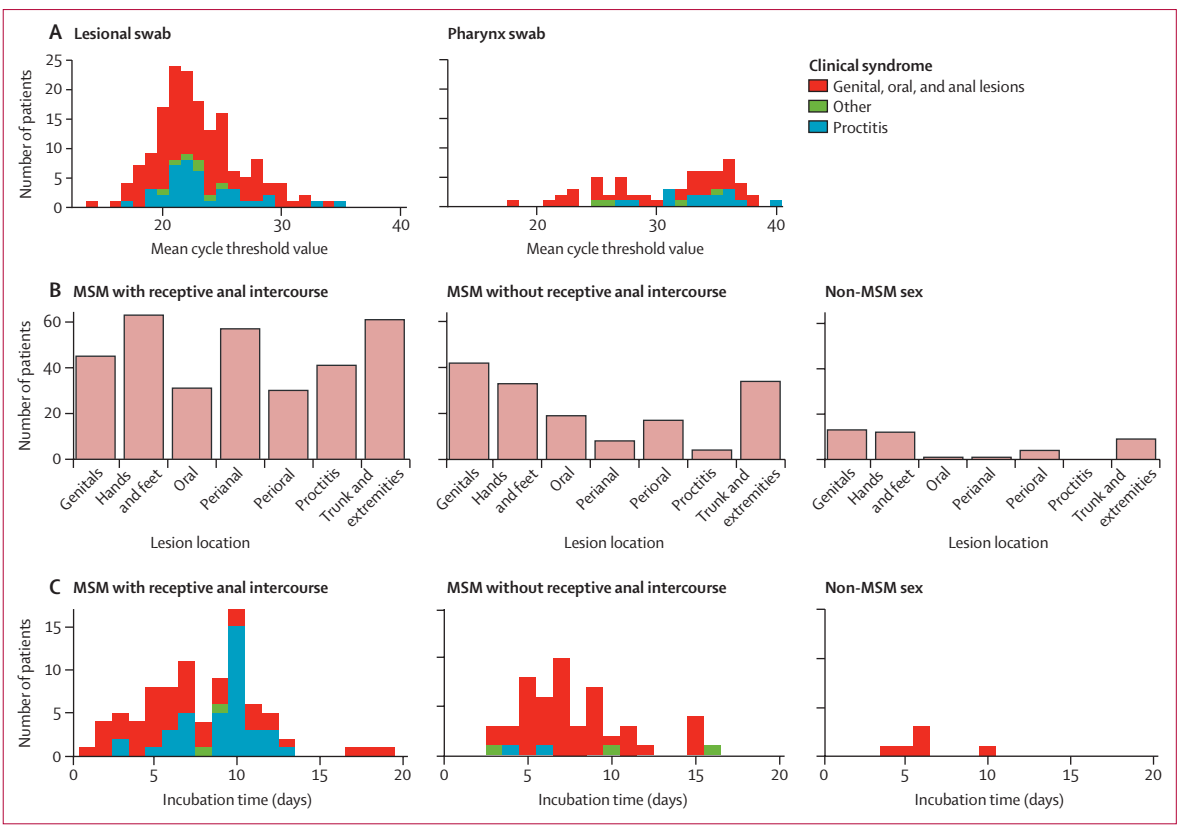

Figure 2: **Mean cycle threshold values by swab location and location of skin lesions and incubation periods by presumed route of infection**
(A) Mean cycle threshold values as a proxy for viral load for lesional swabs compared with pharynx swabs. (B) Location of skin lesions by presumed route of infection. (C) Incubation period of monkeypox by presumed route of infection and location of lesions. MSM=men who have sex with men.

the presence of pathogens in purulent genital lesions that are transmitted through superficial abrasions in the skin or mucous membranes.[20] Anorectal and genital epithelium routes exhibit the highest probability of sexually transmitted infection acquisition because they have a lower degree of keratinisation and a higher frequency of antigen-presenting cells such as macrophages and dendritic cells.[21] Using the PCR cycle threshold as a proxy, we found that viral load in lesions was significantly higher than in pharyngeal swabs. Although imprecise, these findings are consistent with a viral load more than three orders of magnitude higher in lesion samples compared with respiratory samples. This observation, together with the localisation of the lesions, the exposure history of the individuals, and the concurrent sexually transmitted infections, suggests that close contact during sex is the dominant form of monkeypox transmission in the current outbreak. Public health messaging needs to be targeted at appropriate populations who might be at risk and needs to be adapted to highlight the risk of transmission related to close skin-to-skin contact.

Our finding of low viral loads or even negative results in respiratory samples suggests that there might be differences from previous imported cases, which have shown prolonged monkeypox virus DNA detection in swabs of the upper respiratory tract.[11] We speculate that local replication of the virus at the point of entry within lesions of the genital or oral tract might be followed by low-grade or no viraemia, resulting in minimal replication in the respiratory tract and little or no transmission through respiratory droplets. In smallpox, accidental local inoculation or intentional inoculation (ie, variolation) resulted in locally restricted satellite lesions around the point of entry in the absence of disseminated lesions.[5,6]

	MSM with receptive anal contact (n=108)	MSM without receptive anal contact (n=58)	Non-MSM sex (n=15)	Total (n=181)
Incubation period, days	8·0 (5·0–10·0)	7·0 (5·0–9·0)	6·0 (5·0–6·0)	7·0 (5·0–10·0)
Systemic symptoms before the rash	67 (62%)	16 (28%)	4 (27%)	87 (48%)
Presence of proctitis	41 (38%)	4 (7%)	0	45 (25%)
Throat PCR				
Not done	48 (44%)	16 (28%)	0	64 (35%)
Negative	11 (11%)	18 (31%)	6 (40%)	35 (19%)
Positive	49 (45%)	24 (41%)	9 (60%)	82 (45%)

Data are median (IQR) or n (%). MSM=men who have sex with men.

Table 3: **Association between the presumed route of transmission and epidemiological, clinical, and virological factors**

By contrast, generalised poxvirus infections progress in a stepwise manner (with an initial amplification of viral load in the lymph nodes, liver, and spleen), resulting in a high-grade viraemia that leads to disseminated infection of the skin and respiratory tract, and the excretion of infective respiratory droplets.[22–25] Besides the change in the route of transmission, there might be alternative reasons for the localised presentation of monkeypox, such as a novel gain-of-function mutation, that might become evident when more viral sequences are available. Additionally, mild trauma in the pubic, inguinal, and perianal regions during sexual intercourse might cause local vasodilation and a higher density of skin lesions in that particular region (also known as the garter effect).[6]

Our study has some limitations. First, we could not estimate the incubation period in 37 participants because they reported multiple possible exposure events. Second, participants from one of the sexual health clinics did not undergo collection of a throat or anal swab on presentation due to logistical reasons. Third, we only collected samples at diagnosis and did not collect semen samples as part of this study. We are collecting samples, including semen, at multiple timepoints in a currently enrolling study, which might provide more information on viral kinetics (NCT05476744). Similarly, we did not have complete information on skin healing (eg, desquamation of crust and new skin underneath) and had to use the formation of dry crust as the parameter for assessing lesion healing. Finally, blood testing was not routinely done; therefore, we had to infer dissemination from the point of entry to a distant site on the basis of testing of throat swabs, which might have underestimated the number of participants with viraemia. Nevertheless, many participants had samples collected at more than one body site, which enabled us to investigate associations between rash distribution and dissemination of the virus.

Our study strengthens the evidence for skin-to-skin contact during sex as the dominant mechanism of transmission of monkeypox, with important implications for disease control. First, the putative change compared with previous outbreaks in the route of transmission from respiratory to direct contact might promote the spread of the disease through sexual networks. This scenario is similar to previous outbreaks, such as lymphogranuloma venereum L2b, antibiotic-resistant *Shigella*, and hepatitis A, which were transmitted predominantly within sexual networks of MSM.[26] Second, because monkeypox might present with atypical manifestations, clinicians should have a high index of suspicion of the disease, particularly in individuals living in areas with high transmission rates or with potential exposure. Specifically, we describe a proctitis-related clinical syndrome, with different clinical features, including systemic manifestations before lesion onset, in individuals reporting anal-receptive sex, which differs from other presentations. Third, because of the short incubation period, pre-exposure vaccination of groups who are at high risk is likely to be more effective than postexposure vaccination for public health control of the infection. Finally, the strikingly higher viral loads in lesion swabs than in pharyngeal swabs should be further investigated to guide the decision on whether respiratory transmission is relevant and respiratory isolation at home is necessary.

Contributors

EJT-V, CG-Ca, MM, PLO-R, and OM conceived and designed the study. All authors acquired the data. EJT-V, MM, and OM analysed and interpreted the data. EJT-V, MM, and OM did the statistical analysis. EJT-V, AAl, CS, MA-D, MU, CG-Ca, MM, and OM drafted the manuscript. All authors reviewed the manuscript and vouch for the accuracy and completeness of the data and for the adherence of the study to the protocol. All authors were responsible for the final decision to submit for publication. All authors have seen and approved the manuscript. EJT-V, AAl, MU, MM, and OM had full access to all of the data in the study.

Declaration of interests

We declare no competing interests.

Data sharing

De-identified participant data collected for the study, including individual participant data and a data dictionary defining each field in the set, will be made available from the corresponding author on reasonable request.

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