UK Health Security Agency

Mpox routes of transmission

A rapid evidence summary

Contents

Main messages

1. This rapid evidence summary (search up to 29 August 2024) identified and summarised evidence relating to routes of transmission in mpox (Clade Ia, Ib, IIa, IIb) in humans.

2. Twenty-eight studies were included (1 to 28), of which 9 were cross-sectional studies (3, 7, 10 to 12, 18, 19, 27, 28), 9 were prospective cohort studies (2, 4, 5, 15, 17, 20, 23, 25, 26), 7 were retrospective cohort studies (6, 9, 13, 14, 21, 22, 24), 2 were outbreak reports (1, 8), and one was a case-control study (16).

3. Two studies reported on mpox clade I (1, 2), 10 reported on clade II only (3 to 9, 21, 22, 26), one study reported on both clade I and II (10), and 15 studies did not report the clade (11 to 20, 23 to 25, 27, 28). One study on mpox clade I was conducted between November 2021 and January 2022 (1), another on mpox clade not reported was conducted in June 2003 (20). All other studies were conducted between January 2022 and February 2024. It is likely that studies which did not report mpox clade were describing mpox clade IIb, given the countries and timeframes. However, as this was not confirmed the results have been reported separately in this review.

4. One study reported on route of transmission for mpox clade I in suspected and confirmed mpox cases in the current outbreak (January 2023, Democratic Republic of Congo). Twelve cases were identified in the transmission chain from the index case (2). The initial spread was primarily through heterosexual sexual contact. Subsequent transmission routes were heterosexual contact, non-heterosexual contact or non-sexual or undefined contact.

5. Exposure to mpox virus or routes of transmission for mpox clades I, II and clade not reported were investigated in 20 studies (1 to 20). For all clades, sexual contact was the most frequently reported route of transmission in adults in most studies (2, 4 to 9, 11 to 18), and in adolescents aged over 13 years in one study (10), and over 15 years in another study (19). In children, results from 3 studies reported that the most frequent route of transmission in children is direct person-to-person contact (3, 10, 19).

6. Persistence of mpox in semen has been taken in this review as an indicator of potential sexual transmission, and was measured in 6 studies (6, 21 to 25). None of these studies reported on mpox clade I. For mpox clade II, median time to viral clearance of mpox in semen samples was reported as 7 days and 14 days (21, 22), and for mpox clade not reported, median time to viral clearance was reported as being between 8 to 13 days (23 to 25).

7. Three studies measured viral load in surface and air samples (26 to 28). Samples were taken from the hospital rooms of mpox cases in 2 studies (26, 27), and from exhaled breath and air samples in mpox cases in another (28). Evidence from all 3 studies suggested the possibility of airborne transmission, as samples taken from air conditioning outlets, air vents

(26, 27), and exhaled breath and air samples (28) were all positive for mpox virus. The evidence also suggested possible fomite transmission, as samples taken from multiple sites in patient hospital rooms and from personal items were positive for mpox virus (26, 27). However, while surface and air samples were positive for mpox virus, these studies did not investigate onward transmission though air or fomite routes.

8. Critical appraisal was not performed, which restricts the interpretation of the findings, although important limitations of the evidence have been highlighted. Routes of transmission, and whether contacts had symptoms or not was frequently based on self-report and this information may be subjective. While studies report viral detection in semen, surface, or air samples, this may not indicate likelihood of transmission in all cases. Viral load may also vary by stage of infection, but the studies did not all clearly report when surface and air samples were taken.

9. In summary, there was limited evidence to assess routes of transmission in mpox clade I. There were 2 studies, with one study (a preprint) relating to the outbreak in the Democratic Republic of Congo in January 2023. There was more evidence to support route of transmission in mpox clade II or studies where the clade was not reported. The evidence suggested that sexual contact is the most frequent route of transmission in adolescents and adults, while direct person-to-person contact is the most frequent route of transmission in children. Viral load in semen suggested potential for sexual transmission, while viral load in exhaled breath, air, and environmental samples suggested potential for airborne and fomite transmission. However, viral load in these samples is an indirect measure of transmission routes, and no evidence was identified for onward transmission through airborne or fomite transmission.

Purpose

The purpose of this rapid evidence summary was to identify and summarise the available evidence that discussed routes of transmission in mpox in humans.

The review question is:

1. What are the most common routes of transmission for mpox virus (Clade Ia, Ib, IIa, IIb) in humans?

Methods

A rapid evidence summary was conducted, following streamlined systematic methods to accelerate the review process. A literature search was undertaken to look for relevant primary studies published or available as preprint, up to 29 August 2024. A previous review on the infectious and incubation periods, and transmission of mpox was completed by UKHSA in 2022 (29). This was checked for relevant studies.

A protocol was produced before the literature search was conducted, including the review question, the eligibility criteria, and all other methods. Full details of the methodology are provided in the protocol in Annexe A.

One protocol deviation was made to amend the inclusion criteria to include evidence from laboratory confirmed mpox when clade was not reported (from any country), in addition to those specified as clade Ia, Ib, IIa or IIb. This was to ensure that all information on the routes of transmission of mpox was included in this review.

Possible exposures were included where they may suggest potential for transmission. Persistence of mpox in semen has been taken in this review as an indicator of sexual transmission.

There are 2 clarifications to the study protocol:

1. Viral load in semen has been taken as a proxy measure of persistence in semen.
2. Viral load in surface, air, and saliva samples has been taken as a proxy measure of fomite, airborne or droplet transmission respectively.

Viral load was measured using a polymerase chain reaction (PCR) test or cell culture. When measured using PCR, results were reported as the number of PCR copies per millilitre (mL) or cycle threshold (Ct). Ct values are the number of PCR cycles required for viral detection. Lower Ct values reflect a higher viral load. A higher viral load may indicate that a case was more

infectious, and therefore greater likelihood of transmission occurring, although this may not necessarily be true for all cases.

Screening on title and abstracts was undertaken in duplicate by 2 reviewers for 10% of eligible studies, with the remainder completed by one reviewer. Screening on full text was undertaken by one reviewer and excluded studies were checked by a second. Data extraction was performed by one reviewer and checked by a second.

Where available, smallpox vaccination history was reported. For people living with Human Immunodeficiency Virus (HIV), where available, Cluster of Differentiation 4 (CD4) counts were reported.

Evidence

In total, 3,789 studies were screened at title and abstract and 115 studies were identified to be screened at full text. A further 7 studies were identified from a previous review on the infectious and incubation periods and transmission of mpox (29). In total, 122 studies were screened at full text. Of these, 27 studies met the inclusion criteria (2 to 28). One additional study (1) was identified from an mpox review on clinical presentation and severity of mpox, conducted at the same time as this review (31). Therefore, 28 studies were included in this review (1 to 28).

A PRISMA diagram showing the flow of studies through the review is shown in Annexe B, and studies excluded on full text screening are available with the reasons why in Annexe C. Study characteristics are available in Annexe D.

Nine studies were cross-sectional studies (3, 7, 10 to 12, 18, 19, 27, 28), 9 were prospective cohort studies (2, 4, 5, 15, 17, 20, 23, 25, 26), 7 were retrospective cohort studies (6, 9, 13, 14, 21, 22, 24), 2 were outbreak reports (1, 8), and one was a case-control study (16). One study on mpox clade I was conducted between November 2021 and January 2022 (1), another on mpox clade not reported was conducted in June 2003 (20). All other studies were conducted between January 2022 and February 2024.

The studies were conducted in the following countries:

- Argentina, one study (14)
- Belgium, 2 studies (4, 5)
- Brazil, one study (15)
- Central African Republic, one study (1)
- China, 3 studies (9, 18, 26)
- Democratic Republic of Congo, one study (2)
- France, one study (13)
- Italy, 5 studies (6, 21 to 24)
- Netherlands, one study (17)

- Portugal, one study (12)
- Spain, 3 studies (7, 25, 28)
- UK, one study (27)
- United States, 4 studies (8, 16, 19, 20)
- global, 3 studies (3, 10, 11)

Two studies reported on mpox clade I (1, 2), 10 reported on clade II (3 to 9, 21, 22, 26), only one study reported on both clade I and II (10), and 15 studies did not report the clade (11 to 20, 23 to 25, 27, 28).

It is likely that studies which did not report mpox clade were mostly describing mpox clade IIb, given the countries and timeframes. However, as this was not confirmed the results have been reported separately in this review.

Routes of transmission

Twenty studies reported on the route of transmission in mpox (1 to 20, 24), 7 studies in mpox clade IIb (3 to 9), one study that reported mpox clade I and clade II together (10), and 10 studies that did not report mpox clade (11 to 20). A summary of findings from these studies is presented in Tables D.1, D.2, and D.3.

Two studies reported on routes of transmission in mpox clade I (1, 2). One study relates to the outbreak which began in January 2023 in the Democratic Republic of Congo (2), the other reports on an outbreak in Central African Republic in November 2021 to January 2022 (1).

One prospective cohort study reported on route of transmission for mpox clade I in the outbreak which began in January 2023. Mariska and others (published as a preprint) conducted a prospective cohort study in the Democratic Republic of Congo, investigating routes of transmission in the mpox clade Ib outbreak which began in January 2023 (2). Fifty-one suspected or laboratory-confirmed mpox cases were admitted to Kamituga hospital, and transmission links between 13 individuals were examined (54% male, HIV status not known for any cases, age, and vaccination history were not reported). The index case (laboratory-confirmed case) infected 6 other individuals (5 females, one male) in the first link of the transmission chain. Five were infected through heterosexual sexual contact, and one was infected through contact with medical items used to treat the index case. Five of the 6 cases were laboratory-confirmed, while the sixth was symptomatic but not laboratory-confirmed.

The second link of the transmission chain (contact with a case infected by the index case) included 2 cases (both male, laboratory-confirmed) who had heterosexual sexual contact with 2 contacts of the index case (both female, laboratory-confirmed). Two additional cases (both male, symptomatic but not laboratory-confirmed) had either non-sexual contact with a contact of the index case (female, laboratory-confirmed), or undefined contact with a contact of the index case (male, laboratory-confirmed). The third link of the transmission chain included one case

(male, laboratory-confirmed) who had heterosexual contact with a case (female, laboratory-confirmed), and another case (male, symptomatic but not laboratory-confirmed) who had non-heterosexual contact with a case (male, symptomatic but not laboratory-confirmed).

In summary, 12 cases were identified in the transmission chain from the index case. The initial spread was primarily through heterosexual contact (5 out of 6 contacts). Subsequent transmission routes were heterosexual contact (3 out of 6 contacts), non-heterosexual contact (one out of 6 contacts) or non-sexual or undefined contact (2 out of 6 contacts). However, the findings are restricted to one cluster and the sample size in this study is small, restricting the applicability of the findings. This study is summarised in Table D.1.

Besombes and others investigated a clade I outbreak in the Central African Republic in November 2021 (1). Twenty-five confirmed or suspected contacts (median age: 18.5 years [interquartile range (IQR): 5 to 27 years], 28% male, one co-infection with HIV reported, CD4 counts, and vaccination history not reported) were identified from an index case (who was thought to be exposed through animal-to-human transmission after coming into contact with a non-human primate). The study reported on sexual contact as a potential route of transmission between humans, with 16.4% reporting sexual contact with an infected individual. Other reported exposures to mpox from infected individuals were through meal sharing (83.3%), hospital visits (70.8%), or living in the same household (68.4%).

Clade II: routes of transmission

Seven studies evaluated routes of transmission in mpox clade II (3 to 9). One study was global across World Health Organisation (WHO) member states (3), 4 were from cases in Europe (4 to 7), one from the USA (8), and one from China (9). Findings from these studies are summarised in Table D.2.

A global cross-sectional study of 82,807 cases reviewed mpox clade IIb cases identified between January 2022 and January 2023 from the following WHO regions: African regions, Region of the Americas, Eastern Mediterranean region, Southeast Asia region, Western Pacific region (3). HIV status was known for 35,329 cases (48% living with HIV [CD4 counts not reported]. Route of transmission was reported in 21,479 cases (97.2% male, 6.3% men-who-have-sex-with-men (MSM), HIV status and vaccination history not reported for this subset) by age, sex, and sexual orientation. The most frequently reported transmission was through sexual contact (14,941 of 21,479 cases, 68.7%), followed by other undefined routes of transmission (4,012 cases, 18.7%), and direct person-to-person contact (2,374 of 21,479 cases, 11.1%). The most frequent route of transmission in children (0 to 9 years) was reported as direct person-to-person contact, and sexual contact was the most frequently reported in those aged 10 to 17 years of age. This study also reported on vertical transmission during pregnancy or birth, and other exposures including healthcare-associated exposure, occupational exposure, or contact with contaminated material. A full breakdown of routes of transmission or exposures of mpox virus by age, sex and sexual orientation reported in this study is available in Table D.2.

Brosius and others conducted a prospective cohort study of 25 high-risk contacts (defined in this study as sexual contact, skin-to-skin contact for longer than 15 minutes with an mpox case with skin lesions or as living in the same household as an mpox case) from 23 laboratory-confirmed mpox clade IIb cases in Belgium between June and July 2022 (4). The median age of these contacts was 43 years (IQR: 36 to 51 years, 96% MSM), 20% were living with HIV (treatment status and CD4 counts not reported), and 44% had a history of childhood or post-exposure smallpox vaccination (6 participants received post-exposure vaccination). Of these 25 high-risk contacts, 18 (72%) reported having sexual contact with a laboratory-confirmed case, and 7 (28%) had non-sexual contact with a laboratory-confirmed case. Participants were followed up for a median of 16 days (IQR: 14 to 26 days) after their last high-risk contact. Twelve (67%) of the 18 contacts who reported sexual contact with a laboratory-confirmed case were subsequently infected (Ct value of less than 37), while none of the 7 contacts who reported non-sexual contact were subsequently infected ($p = 0.03$).

A second prospective cohort of 155 men with mpox clade IIb from Belgium was also reported on between May and September 2022 (5). The median age was 39 years (IQR: 33 to 46 years), with 95.5% MSM, 34.2% were living with HIV (12.4% had a CD4 count lower than 500 cells per microlitre [µL]), 18% were vaccinated against smallpox or mpox. Of the 155 men, 37 reported having contact with a suspected or confirmed case 3 weeks prior to symptom onset, with 30 reporting sexual contact, 7 reporting household exposure, skin-to-skin or non-touch contact (within 1.5m), and 118 reporting no contact at all. One-hundred and forty-five of the 155 were sexually active, with the following sexual practices: anal-insertive (92 out of 145 cases, 63.4%), anal-receptive (95 out of 145 cases, 65.5%), oral (69 out of 145 cases, 47.6%), and vaginal (8 out of 45 cases, 5.5%).

Mazzotta and others reported on a cohort of 541 mpox clade IIb cases (99.3% male, 74.7% Caucasian, median age 38 years [IQR: 33 to 44 years], 94.6% were gay or bisexual) from 15 health centres in Italy between May and September 2023 (6). Of this cohort, 235 (43.4%) were living with HIV (4.1% with a CD4 count less than 350 cells per µL), and 61 (11.3%) reported previous smallpox vaccination. Transmission routes were not stratified by HIV status or vaccination history. Of the whole cohort, 502 (92.8%) reported sexual transmission, while 39 (7.2%) reported non-sexual contact.

Inigo Martinez and others reported on routes of transmission in 508 mpox clade IIb cases (99% male, median age: 35 years [IQR: 12 years], 93% MSM, 44.3% living with HIV, vaccination history not reported) in Spain in May and June 2022 (7). Forty-five clusters with 96 linked cases were identified, ranging from 2 to 4 cases per cluster. There were 19 transmission chains identified between 42 mpox cases, made up of 20 primary cases, 21 secondary cases and one tertiary case. All secondary cases were exposed during close physical contact during sexual activity (13 secondary case exposures were between household members, 8 between non-household members).

Leonard and others interviewed 56 laboratory-confirmed mpox clade IIb cases in the USA (56% male) to identify exposure and route of transmission between May and August 2023 (8). The

median age of cases was 35 years (IQR: 26 to 42 years), 80% of cases were gay or bisexual, 30% were living with HIV (8.3% CD4 counts less than 350 cells per millimetre [mm]^3), 43% were partially or fully vaccinated against mpox. Of the 56 cases, 55 were interviewed. Seven reported undefined contact with someone with mpox symptoms in the 3 weeks prior to symptom onset. Two pairs of cases (positive for mpox) disclosed sexual contact with another case (confirmed positive for mpox) in the 3 weeks preceding symptom onset.

Dou and others reported on routes of transmission in 37 laboratory-confirmed mpox clade IIb cases in China in May and June 2023 (9). All participants were men, aged between 24 and 51 years (median age: 30 years [IQR: 26.5 to 34.5 years]), and 36 of the 37 cases identified as MSM. Nineteen of the 37 (51.4%) were living with HIV (CD4 counts not available for all patients), and 3 (8.1%) had history of smallpox vaccination. Thirty-three close contacts (sexual partners, family or household members who have had direct or indirect skin or mucous membrane contact, or had shared a confined space with a case for an extended period of time) who had contact with a confirmed cases within 4 days of symptom onset were identified (18 regular or casual male sexual partners, 6 family members, 6 roommates and 3 healthcare workers [HCWs]). Of these, 6 (18.2%) subsequently tested positive for mpox either upon detection or 7 days after their last exposure. Transmission was only recorded in individuals who had sexual contact with a confirmed case, no other transmission routes between family members, households or HCWs were observed.

Clade I and II: routes of transmission

One cross-sectional study reported on routes of transmission of mpox (clade I and II) in 1,118 children and adolescents under 18 years old with confirmed infection between January 2022 and May 2023 where cases were notified to WHO in the following WHO regions: Region of Americas (61.8%), African Region (30.3%), European Region (7.5%), Eastern Mediterranean Region (less than 1%), and Western Pacific Region (less than 1%) (10). Of 1,102 cases where case information was known, 58.5% were male, 82.2% had unknown sexual behaviour, and 1% were living with HIV (68.5% unknown HIV status). Vaccination history was not reported. Virus clade was assumed by the authors of this study based on reporting country or sub-national area of circulating clades. The study authors assumed 297 cases of mpox clade I (98 cases in 0 to 4 year olds, 144 cases in 5 to 12 year olds, 55 cases in 13 to 17 year olds), and 805 cases of mpox clade II (224 cases in 0 to 4 year olds, 208 in 5 to 12 year olds, 373 cases in 13 to 17 year olds). Data on transmission route were not separated by clade or region.

In 0 to 4 year-olds (data available for 28 cases), the most frequent route of transmission was direct person-to-person contact (39.3%). Other potential exposures and routes of transmission were through contact with contaminated material (21.4%), healthcare-associated exposure (3.6%), vertical transmission during pregnancy and birth (3.6%), and other (undefined) exposures (32.1%). In 5 to 12 year olds (data available for 25 cases), transmission of mpox virus was mainly through direct person-to-person contact (40.0%), followed by exposure by contact with contaminated material (24.0%) and other exposures (36.0%). In 13 to 17 year olds (data available for 64 cases), the most frequent route of transmission was through sexual

contact (53.1%), followed by direct person-to-person contact (18.8%). Other potential exposures in 13 to 17 year olds were contact with contaminated material (4.7%), through healthcare (3.1%), and through other exposures (20.3%). HIV and immunosuppression status for each age group is presented in Table D.2, however data was not presented separately by these.

Clade not reported: routes of transmission

Ten studies reported route of transmission, but not mpox clade (11 to 14, 16, 18 to 20, 23, 25). One study was global (11), 3 studies were conducted in Europe (12, 13, 17), 2 in South America (14, 15), 3 in North America (16, 19, 20), and one in Asia (China) (18). A summary of findings from these studies is presented in Table D.3.

Angelo and others reported on transmission routes in 226 cases of mpox from 15 countries (Spain, Canada, Germany, France, Belgium, Netherlands, Portugal, Sweden, Romania, USA, Israel, South Africa, UK, Denmark, Argentina) reported between May and July 2022 (11). All included cases were male, with a median age of 37 years (IQR: 32 to 43 years), 98% were MSM, 44% were living with HIV (92% had undetectable HIV load), median CD4 count: 713 cells per mm^3 (IQR: 500 to 885 cells per mm^3), and 9% had history of previous smallpox vaccination). Of 195 patients with available information, 78 (40%) reported contact with a suspected or confirmed mpox case. The most frequently reported was sexual or close intimate contact (99%), followed by household contact (11%), face-to-face contact outside of household (3%), or other contact (4%). However, all patients with household contact also reported sexual contact, and all those with other forms of contact were in a couple with a confirmed case.

Seven other studies reported that, in adult participants, sexual contact was the most frequently reported route of transmission (11.1% to 95.0% of participants, where transmission route was known) (12 to 18). Household contact, non-sexual contact, and other direct but non-sexual contact were also frequently reported. Prolonged face-to-face contact was reported in one study for 2.0% of participants (17), and one participant in one study reported needle sharing with a confirmed case (12). The majority of these studies were conducted between May and October 2022, with the exception of Snyder and others (November 2022 to June 2023) (16) and Zong and others (May to July 2023) (18). Sample sizes, country, demographic information about the mpox cases, their reported transmission routes and proportion of participants reporting exposures or route of transmission in each of these studies are presented in Table D.3.

Hennessee and others reported exposure setting and routes of transmission in 83 mpox cases in children and adolescents aged under 18 years in the USA (May to June 2022) (80% male at birth [one transgender male], HIV status not reported) (19). Exposure setting and route of transmission was reported by age group; 0 to 4 year olds (n=16), 5 to 12 years (n=12), and 13 to 17 year olds (n=55). In both the 0 to 4 and 5 to 12 year age groups, household exposure was the most frequently reported (81% and 50% respectively). In those aged 13 to17 years, sexual contact was the most frequently reported (62%, over 15 years of age). No 13 to 17 year olds reported households as the exposure setting. Cases as a consequence of household contact were mostly through direct skin-to-skin contact that routinely occurred between a child and adult

caregiver, although in one case fomite transmission was suspected. There were 2 instances of the adult caregiver contracting mpox through skin-to-skin contact during routine childcare. The study did not identify any evidence of secondary transmission when children attended school or childcare facility while symptomatic.

One study in the USA (June 2003) monitored HCWs after they were exposed to a confirmed mpox case (20). There were 57 HCWs (74% female, median age: 39 years [range: 19 to 61 years], 31% had history of smallpox vaccination, HIV status was not reported) who reported the following exposures:

- same air: 52 of 57 HCWs (91%)
- same room: 52 of 57 HCWs (91%)
- skin-to-skin contact: 28 of 57 HCWs (49%)
- contact with patient belongings: 46 of 57 HCWs (81%)

Unprotected exposure (exposure without personal protective equipment [PPE]) was reported by 70% of the exposed HCWs. While most (35 of 57 exposed HCWs [61%]) reported using gloves for every patient encounter, the use of gowns (19 of 57 exposed HCWs [33%]), surgical masks (14 of 57 exposed HCWs [25%]), and N95 respirators (11 of 57 exposed HCWs [19%]) for every patient encounter was less common. None of the exposed HCWs became symptomatic. One tested positive for anti-orthopoxvirus IgM in serum samples, but this was suspected to be due to recent smallpox vaccination, rather than exposure to an mpox case. The use of PPE may have reduced the risk of infection; however, the results of this study suggest a reduced likelihood of airborne or fomite transmission in those who have been directly exposed.

Summary: routes of transmission

For all clades (clade I, II and clade not reported) sexual contact was the most frequently reported route of transmission in adults (2, 4 to 9, 11 to 18). In adolescents, it was reported that sexual contact was the most frequent route of transmission in 13 to 17 year olds (10), and in 15 to 18 year olds (19). In children, results from 3 studies suggest that the most frequent route of transmission in children is direct person-to-person contact (3, 10, 19), with 2 studies reporting that the next most frequent route of transmission is through contact with contaminated objects in those aged under 12 (reported as aged 0 to 4 years and 5 to 12 years (10), and those aged 0 to 9 years (3)).

One study assessed presence of symptomatic infection following airborne or fomite exposure (20). No instances of transmission were recorded in this study.

No studies stratified transmission route by HIV or vaccination history. Route of transmission was often self-reported, with many studies frequently reporting an unknown route of transmission.

Eight of the 10 studies that reported on exposure or routes of transmission where mpox clade was not reported were conducted while there were ongoing mpox clade IIb outbreaks in their country, and so it is likely that cases are clade IIb (12 to 19).

Persistence in semen

Six studies measured the persistence of mpox virus in semen (6, 21 to 25), (3 in mpox clade IIb (6, 21, 22), 3 in mpox clade not reported (23 to 25)). A summary of findings from these studies is presented in Table D.4.

Mpox clade II: persistence in semen

Three retrospective cohort studies were identified that evaluated the persistence of mpox clade IIb in semen (6, 21, 22). All followed cohorts in Italy between May and December 2022.

Meschi and others measured viral load in semen samples up to 4 weeks following laboratory-confirmed diagnosis (21). Eighty-nine participants (41.6% living with HIV [median CD4 count 560.5 cells per mL^3, IQR: 412 to 797.3 cells per mL^3], 4.5% had history of smallpox vaccination, ethnicity and sexual orientation not reported) were enrolled between May and December 2022. One hundred and twenty-two semen samples were taken from this cohort over 4 weeks. The highest percentage of samples positive for mpox was at week one (64% of 40 samples) and week 2 (74% of 42 samples), with lower percentages of positive samples at week 3 (38% of 21 samples) and week 4 (32% of 19 samples). The proportion of samples positive for mpox was statistically significantly higher at week one compared to week 4 ($p<0.001$), and the proportion of samples testing positive for mpox from week 2 was statistically significantly higher than both week 3 ($p<0.05$) and week 4 ($p<0.005$). The median Ct value was 38.3 (95% Confidence Interval [CI]: 34.2 to over 40), and median time to viral clearance in semen samples was 14 days (95% CI: 13 to 17 days). Data was not stratified by HIV status or vaccination history.

This study also assessed the presence of infectious virus (and so potentially increased likelihood of transmission) by recovering infectious (replication-competent) virus in 11 semen samples (median Ct value: 27.9 [IQR: 25.2 to 29.5]; median days since symptom onset: 10 [IQR 7.5 to 11.5 days]) from 10 patients. Replication-competent virus was successfully isolated from 2 of these semen samples (from different patients). The Ct values for the positive isolates were 22.7 and 29.3, with the days since symptom onset being 4 and 12, respectively.

Piralla and others also measured viral load in semen samples from participants recruited between May and September 2022 (22). Samples were taken from a cohort of 353 individuals (99.2% male, median age: 37 years, [range: 15 to 67 years, IQR: 32 to 43 years], 10.5% living with HIV, 65.4% previously received smallpox vaccination). At diagnosis, 37 of 77 semen samples taken (48.1%) were positive for mpox. Time to viral clearance was evaluated in a subset of patients, and in 24 samples of semen, median time to viral clearance was 7 days. Replication-competent virus was isolated in 100% (3 out of 3) seminal specimens, supporting

the evidence of infectiousness of mpox virus in semen. Data was not stratified by HIV status or vaccination history.

Persistence of mpox in semen after symptom resolution was evaluated in one study (6). In a cohort of 541 cases (99.3% male, 74.7% Caucasian, median age 38 years [IQR: 33 to 44 years], 43.4% living with HIV [4.0% had CD4 counts less than 350 cells per µL, 94.6% were gay or bisexual) recruited between May and September 2022, 28 semen samples were tested. After symptom resolution, 12 (42.9%) of these semen samples were still positive and some remained positive up to 46 days after symptom resolution, with a mean Ct value range of 31.7 to 40.6

Clade not reported: persistence in semen

One retrospective (24) and 2 prospective (23, 25) cohort studies measured persistence of mpox in semen in cases where mpox clade was not reported. All were conducted in Europe. Two were conducted between May and October 2022 (23, 25), and one between May and November 2023 (24).

Candela and others measured viral load of mpox Deoxyribonucleic acid (DNA) in semen samples of 140 mpox cases in Italy, between May and October 2022 (23). Of the 64 samples available at baseline, 43 (67%) were positive for mpox (median Ct value: 34 [IQR: 31 to 36]). The median age of these cases was 36 years (IQR: 34 to 42 years), 98% MSM, 28% living with HIV, ethnicity and vaccination history not reported. During the 6-month follow-up, mpox viral load in semen was reassessed for 32 out of 43 participants (74%) who had previously tested positive. Median time to viral clearance was 10.5 days (IQR: 7 to 33 days). The proportion of samples negative for viral DNA was 68% (19 out of 28 samples) one week after the baseline measurement was taken, 89% (25 out of 28 samples) after 2 weeks, 90% (26 out of 28 samples) after 3 months, and 100% (32 out of 32 samples) at 6 months.

Raccagni and others measured detectable mpox virus in semen samples in 95 mpox cases in Italy, between May and November 2023 (24). The median age of the cohort was 39.4 years (IQR: 35.4 to 44.7 years), 100% were MSM, 52.6% were living with HIV (89.8% had HIV-RNA less than 50 copies per ml, CD4 count 690 cells per µL [IQR: 559 to 1,005 cells per µL], and 16.8% reported previous smallpox vaccination. The median number of days where mpox virus was detectable in semen was 8 days (IQR: 7 to 15 days)

Suner and others measured time to viral clearance in semen samples from 77 mpox cases (median age: 35 years [IQR: 29 to 46 years], 91% MSM, 51% living with HIV, 59% European) in Spain (June and September 2022) (25). The proportion of semen samples positive for mpox at the following timepoints was:

- one to 5 days: 63% (95% CI: 41% to 86%)
- 6 to 10 days: 71% (95% CI: 50% to 86%)
- 11 to 15 days: 44% (95% CI: 14% to 79%)
- 16 to 20 days: 23% (95% CI: 11% to 44%)

- 21 to 25 days: 32% (95% CI: 18% to 50%)
- more than 25 days: 4% (95% CI: 1% to 10%)

The median time to viral clearance in semen from symptom onset was 13 days (95% CI: 9 to 18 days), and time to viral clearance was approximately 13 days in those living with HIV, and 19 days in those who were not living with HIV, which was statistically significantly shorter (p=0.0043). However, 38 of the 39 mpox cases living with HIV in this study were taking antiretroviral medication for HIV, and 34 (87%) had undetectable viral load. Three samples (1%) had a viral load of 6.5 $\log_{10}$ copies per mL or higher, which is indicative of a higher transmission risk. It took 2 days (95% CI: 0 to 11 days) for viral load to fall below 6.5 $\log_{10}$ copies per mL in 90% of patients, and 8 days (95% CI: 0 to 19 days) for 95% of patients.

Summary: persistence in semen

For mpox clade IIb, median time to viral clearance was reported as 7 days and 14 days in 2 studies (21, 22). For mpox clade not reported, median time to viral clearance was reported as between 8 and 13 days (23 to 25), with one study reporting that time to viral clearance was statistically significantly shorter in those living with HIV compared to those living without HIV (25). However, the majority of mpox cases in this study were on treatment for HIV, and 34 had undetectable viral load.

Time to viral clearance was taken as a proxy measure of persistence of mpox in semen, which can lead to sexual transmission.

For the 3 studies where mpox clade was not reported, it is likely that cases in these studies are mpox clade IIb, as there was an outbreak of mpox clade IIb in both Italy and Spain during the reported study dates.

Evidence from another rapid evidence summary conducted by UKHSA on mpox incubation and infectious period, also included studies reporting persistence in semen and provides a comparison of semen samples over time to other samples taken from people with mpox (for example, throat, skin, blood, urine, or rectal sample) (21, 23 to 25, 30). When compared to other sample types, that review showed that although all studies (mpox clade IIb and where clade not reported) report shorter median time to viral clearance in semen samples, semen samples had the highest percentage of samples still testing positive at 4 weeks in one study (21) and another showed that viral load was still detectable at 22 days or more in semen and skin samples only (25).

Studies evaluating the persistence of mpox in semen were limited by small sample sizes, as often only small subsets gave samples at each time point. Additionally, it was not always clear in the included studies if samples were taken from the same participant at sequential time points, or if samples were from different cases at each time point.

Environmental sampling

Three studies measured viral load of mpox in environmental samples (one in mpox clade IIb (26), and 2 where mpox clade was not reported (27, 28)). A summary of findings from these studies is presented in Table D.5.

Clade II: environmental sampling

One prospective cohort study, conducted in China between June and November 2023, took 1,633 samples from the hospital rooms of confirmed mpox clade IIb cases (100% male, median age 30 years [IQR: 21 to 51 years], 93.5% MSM, 54.5% living with HIV, median CD4 count: 450 [IQR: 237 to 566]) (26). Samples were taken from a range of locations in patient hospital rooms (air conditioning outlet, floor, bedside cupboard, bed handrail, chair, call button, light switch, delivery window, and shower, toilet, and door handles), and from patient's personal items (mobile phone, clothes, television remote, pillows). In total, 860 (52.7%) of 1,633 samples were positive for mpox, with a mean viral load of 5.37 $\log_{10}$ copies per mL (Ct value: 32.83). Mean viral loads were highest in the air conditioning outlet (5.82 $\log_{10}$ copies per mL), and this was also the area with the highest proportion of swabs positive for mpox (65 [69.9%] of 93 samples positive for mpox). The other areas with the highest proportion of swabs positive for mpox were pillow samples (85 [68%] of 125 samples positive for mpox), floor samples (56 [62.9%] of 89 samples positive for mpox), and samples taken from bedside cupboard (77 [61.6%] of 125 samples positive for mpox). Proportion of positive samples from all areas sampled is presented in Table D.5.

This study also reported on the median viral loads and the distribution of mpox viral loads on surfaces in patient rooms by days from symptom onset (0 to 7 days, 8 to 14 days, 15 to 21 days). Across all sites, median viral load ranged from 4.69 to 5.57 $\log_{10}$ copies per mL for 0 to 7 days after symptom onset, from 4.84 to 5.72 $\log_{10}$ copies per mL for 8 to 14 days after symptom onset, and 4.89 to 6.01 $\log_{10}$ copies per mL for 15 to 21 days after symptom onset. There was no statistically significant difference for median viral load between 1 to 7 days post-symptom onset, 8 to 14 days post-symptom onset or 15 to 21 days post-symptom onset (all $p > 0.05$). The proportion of samples positive for mpox with viral loads higher than 6.59 $\log_{10}$ copies per mL (and so more indicative of transmission risk) was also reported and was highest for the deposition area (17.2%), floor (11.8%), bedside cupboard (11.2%), and pillow (10.4%) (which were also the areas with the highest proportion of swabs positive for mpox). A full breakdown of median viral loads (and IQR), and the proportion of samples taken that were positive for mpox with viral loads higher than 6.59 $\log_{10}$ copies per mL, positive for mpox with viral loads lower than 6.59 $\log_{10}$ copies per mL, and negative for mpox by area and time from symptom onset is presented in Table D.5.

Mpox clade not reported: environmental sampling

Two cross-sectional studies measured surface, air, or saliva samples contaminated by mpox cases (27, 28). Both were conducted in Europe, between May and September 2022.
Gould and others measured surface and air samples in respiratory isolation rooms of 7 hospitalised mpox cases in the UK (May to June 2022), who had active skin lesions (ethnicity, age, sex, HIV status and sexual orientation of cases were not reported) (27). Surface samples were taken from areas highly likely to have been touched by the patient (isolation room floor, call button, light switch, tv remote, observation machine, window ledge, chair, and door, toilet flush and shower handles, as well as tap handles in the bedroom and bathroom) and from areas unlikely to be touched by the patient (bathroom vent, anteroom floor, corridor floor). In areas likely to be touched by mpox patients, between 80 to 100% of samples were positive for mpox, with Ct values ranging from 24.7 to 37.4. In areas unlikely to be touched by mpox patients, between 40 and 100% of samples were positive for mpox, with Ct values ranging from 25.9 to 37.5. One hundred percent of samples taken from the bathroom vent were positive for mpox (Ct values: 25.9 to 33.6), which the authors highlight as being suggestive of airborne transmission, as this area is the only area in the patient room not touched by mpox cases.

Air samples and samples taken from PPE were also measured. Air samples were taken from within the patient room before and during bedding change, and from the corridor and anteroom before and after PPE change. The following proportion of positive air samples and Ct values were reported:

- patient room (within 1m of bed), before bedding change: 0 out of 5 samples positive for mpox
- patient room (within 1m of bed), during bedding change: 2 out of 5 samples positive for mpox (Ct values: 32.7 and 36.2)
- patient room (more than 1.5m from bed), before bedding change: 2 out of 5 samples positive for mpox (Ct values: 36.2 and 36.5)
- patient room (more than 1.5m from bed), during bedding change: one out 5 samples positive for mpox (Ct value: 35.8)
- corridor, before putting on PPE: one out of 3 samples positive for mpox (Ct value: 38.2)
- corridor, while putting on PPE: 0 out of 5 samples positive for mpox
- anteroom, before putting on PPE: 0 out of 5 samples positive for mpox
- anteroom, while putting on PPE: 0 out of 5 samples positive for mpox

Twelve samples were taken from PPE (including gloves, gowns, and visor), of which 4 (33.3%) were positive with Ct values ranging 26.1 to 35.6.

Hernaez and others collected exhaled breath, air, and saliva samples from 44 men with symptomatic mpox (median age 35 years, IQR: 11.3 years, 94% MSM, 52% living with HIV [100% had undetectable viral load], 25% received smallpox vaccine) in Spain, between May to September 2022 (28). Exhaled breath samples were taken from filters in the interior of an FPP2

mask, and 32 (71%) out of 45 exhaled breath samples were positive for mpox, (lowest Ct value recorded: 26). Air samples were taken from 2m to 3m away from and 1.5m above the patients, while the patient breathed through a face mask. Of 42 air samples, 27 (64%) were positive for mpox (despite participants wearing a FFP2 face mask during testing), and the lowest Ct value recorded was 29. Of 41 phlegm-free saliva samples, 35 (85%) tested positive for mpox, and the lowest Ct value recorded was 18.

Summary: environmental sampling

Evidence from all 3 studies suggested possible airborne transmission, as samples taken from areas such as air conditioning outlets and air vents (26, 28) and exhaled breath and air samples (28), were positive for mpox. The evidence is also suggestive of fomite transmission as samples taken from multiple sites in patient rooms and from personal items were positive for mpox (26, 28), and droplet transmission, as a high proportion of saliva samples was positive for mpox (28). However, the studies did not assess onward transmission though air or fomite transmission.

While clade was not reported in 2 studies, these studies were conducted while there was a known clade IIb outbreak in both the UK and Spain, and so included cases are also likely to be mpox clade IIb.

Health inequalities

Many of the studies included people who were living with HIV, however their antiretroviral treatment status or CD4 counts were not consistently reported, and it was unclear if they were immunocompromised. Evidence for transmission route or persistence in semen was mostly not stratified by HIV status, so difference between those who are living with HIV, those who are living with HIV but have undetectable levels of virus, and those who do not have a diagnosis of HIV cannot be clearly established.

Three studies evaluated transmission routes in children, in mpox clade II (3), clade I and II (10), or mpox clade not reported (19). These studies identified that routes of transmission are different in children compared to adolescents or adults, with all studies reporting that direct person-to-person contact was the most frequent route of transmission in these groups (3, 10, 19). Studies in adults all reported sexual transmission as the most frequent route of transmission in their cohorts (2, 4 to 9, 11 to 18), as did studies of adolescents aged over 13 years (10), and 15 years (19).

No evidence was identified in those who are pregnant or other vulnerable groups pre-defined as being at high risk of health inequalities in the review protocol. This rapid evidence summary therefore does not provide further information on health inequalities in the groups identified as being at high risk with respect to this review question.

Limitations

This rapid evidence summary used streamlined systematic methods to accelerate the review process. Sources of evidence searched included databases of peer-reviewed and preprint research, but an extensive search of other sources was not conducted and most article screening was completed without duplication, so it is possible relevant evidence may have been missed.

To ensure rapid completion of this work, critical appraisal was not performed. This limits the interpretation of the findings, although important limitations of the evidence have been highlighted in this report.

Route of transmission is usually self-reported by the mpox case, and so is subjective and may be inaccurately recalled.

Viral load and viral positivity, used as a proxy for persistence in semen and airborne and fomite transmission, are indirect measures of transmission as while viral load in semen, air, saliva, or surface samples may be suggestive of sexual, airborne, droplet or fomite transmission respectively, it does not represent onward transmission. Higher viral loads or a positive test may indicate a higher likelihood of transmission, though this may not be true for all cases.

For studies that have reported on persistence of semen, these were limited by small sample sizes as often only small subsets gave samples at each time point. Additionally, it is always clear in the included studies if samples were taken from the same participant at sequential time points, or if samples were from different cases at each time point.

For studies that have reported on viral load in surface and air samples, it is not always clear when viral load samples have been taken. Ct values may also be influenced by stage of infection (high viral load is usually detected at the early stage of infection and decreases as the infection progresses), however this was not consistently reported between studies.

The limitations identified in this review impact the level of confidence in its findings, potentially influencing the overall reliability and interpretation of the results.

Evidence gaps

For mpox clade I, 2 studies were identified that assessed route of transmission (one in the outbreak that began in January 2023). No studies were identified for the persistence of semen or presence of mpox in air or surface samples for mpox clade I. No studies reported on route of transmission of mpox clade I in children or adolescents.

Limited evidence was identified for airborne, droplet or fomite transmission.

Conclusion

This rapid evidence summary identified 28 studies that reported on exposure to mpox virus or routes of transmission in mpox; 20 studies reported on route of transmission, 5 reported on the persistence of mpox in semen and 3 reported on the presence of mpox virus in environmental samples from the surface and air.

Two studies reported on route of transmission of mpox clade I, with one reporting on the outbreak in the Democratic Republic of Congo that began in January 2023. In this study, the most frequently reported route of transmission was sexual contact (first heterosexual, then homosexual).

Nineteen studies reported on exposure to mpox virus or route of transmission in a mixed sample of mpox clade I and II, clade II only or mpox clade not reported. In these studies, sexual contact was the most frequently reported route of transmission in adolescents and adults, and direct contact was the most frequent route of transmission in children.

Persistence of mpox in semen was measured in 5 studies (3 mpox clade II, 2 mpox clade not reported). For mpox clade II, median time to viral clearance was reported as 7 days and 14 days in 2 studies, and when mpox clade was not reported, median time to viral clearance was reported as between 8 to 13 days.

Three studies measured viral load in surface and air samples. Samples were taken from the hospital rooms of mpox cases and from exhaled breath and air samples in mpox cases. Evidence from all 3 studies is indicative of the possibility of airborne transmission, as samples taken from air conditioning outlets, air vents and exhaled breath and air samples were positive for mpox virus. The evidence is also indicative of fomite transmission, as samples taken from multiple sites in patient rooms and from personal items were positive for mpox virus. However, while surface and air samples were positive for mpox virus, there is no evidence of onward transmission though air or fomite routes in these studies.

Critical appraisal was not performed, which restricts the interpretation of the findings, although important limitations of the evidence have been highlighted. Routes of transmission, and whether contacts had symptoms or not was frequently based on self-report and may be subjective. It should also be noted that while studies report viral load in semen, surface, or air samples and this may indicate likelihood of transmission, this may not be true for all cases. Viral load may also vary by stage of infection, but the studies did not all clearly report when surface and air samples were taken. HIV positivity, treatment and CD4 counts were inconsistently reported between studies, therefore it was not possible to determine from the evidence whether route of transmission was affected by HIV positivity or immune status of the cases.

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UKHSA's rapid reviews and evidence summaries aim to provide the best available evidence to decision makers in a timely and accessible way, based on published peer-reviewed scientific papers, and papers on preprint servers. Please note that the reviews:

- use accelerated methods and may not be representative of the whole body of evidence publicly available
- have undergone an internal independent peer review but not an external peer review
- are only valid as of the date stated on the review

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21. Meschi S and others. 'MPXV DNA kinetics in bloodstream and other body fluids samples' Scientific Reports 2024: volume 14, issue 1, page 13,487
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Annexe A. Protocol

Review question

The review question is:

1. What are the most common routes of transmission for Mpox (Clade Ia, Ib, IIa, IIb) in humans?

A search for primary evidence to answer this review question will be conducted up to 29 August 2024.

Eligibility criteria

Table A.1 Inclusion and exclusion criteria

	Included	Excluded
Population	Humans (any age): • children (aged up to and including 16 years) • adults	Animals
Settings	Any	
Intervention or exposure	Laboratory-confirmed infection with any clade of Mpox (Clade Ia, Ib, IIa, IIb) Or: Clinically-suspected or laboratory-confirmed infection with mpox (Clade Ia, Ib, IIa, IIb, or unspecified) in Clade I outbreak countries (DRC, Republic of Congo, Central African Republic, Burundi, Rwanda, Uganda, Kenya, Cameroon, Gabon) since 1 January 2023	
Outcomes	Any measure of human-to-human transmission through the following routes: • airborne • droplet • fomite • direct contact • sexual (including persistence in semen)	• animal-to-human transmission • transmission through other routes: ingestion, vector-borne

	Included	Excluded
Language	English	Any other language
Date of publication	Up to 29 August 2024.	
Study design	• experimental studies: randomised-controlled trials, quasi-experimental studies, cross-over designs, before-and-after studies • observational studies: cross-sectional, case-control, and cohort studies	• systematic or narrative reviews • modelling studies • case reports • case series
Publication type	Peer-reviewed published research Preprints	Editorials Letters News articles Grey literature Conference abstracts

Identification of studies

The following databases will be searched for studies published up to 29 August 2024: Ovid Medline, Embase, and Web of Science Preprint Citation Index. The search strategy is presented below.

A previous review on the infectious and incubation periods, and transmission of mpox was completed in 2022 (29). This will be checked for relevant studies.

Screening

Title and abstract screening will be undertaken in duplicate by 2 reviewers for at least 10% of the eligible studies, with the remainder completed by one reviewer. Disagreement will be resolved by discussion or with involvement of a third reviewer where necessary.

Screening on full text will be undertaken by one reviewer and checked by a second.

Data extraction

Summary information for each study will be extracted and reported in tabular form. Information to be extracted will include country, study period, study design, participants, results, and any relevant contextual data. This will be undertaken by one reviewer and checked by a second.

Risk of bias assessment

Risk of bias of included studies will not be assessed in this rapid evidence summary due to time constraints.

Synthesis

Where studies are similar enough to combine and present data in a consistent format, a narrative synthesis will be produced to interpret the findings. The number of studies, the number of participants in each study, effect size and variance and a summary of study limitations across studies reporting each outcome will be summarised and presented. Alternatively, if studies present methodological differences that would make synthesis inappropriate, a narrative summary of each study will be provided.

Health inequalities

Variations across the following populations and subgroups will be considered, where evidence is available: those who may be at high risk from mpox including pregnant women and those who are immunocompromised.

Search strategy

Ovid MEDLINE(R) ALL (1946 to 29 August 2024)

1. "Mpox (monkeypox)"/ (2754)
2. Monkeypox virus/ (1420)
3. Poxviridae Infections/ or Poxviridae/ (3692)
4. monkeypox.tw,kf. (4185)
5. monkey pox.tw,kf. (124)
6. mpox*.tw,kf. (1730)
7. monkeypoxvir*.tw,kf. (13)
8. hMPXV*.tw,kf. (28)
9. MPXV*.tw,kf. (855)
10. MPX*.tw,kf. (1398)
11. chimpanzeepox.tw,kf. (1)
12. chimpanzee pox.tw,kf. (0)
13. or/1-12 (8882)
14. (transmi* adj5 (route* or mode or modes or path or paths or pathway* or method* or way* or how or direct* or indirect* or primary or secondary or pattern* or vehicle* or window*)).tw,kf. (64483)
15. transmi*.ti,kf. (142232)
16. exp Disease Transmission, Infectious/ (83190)

17. "Person to person".tw,kf. (4427)
18. "human to human".tw,kf. (7097)
19. contact*.tw,kf. (504058)
20. "Skin to skin".tw,kf. (8136
21. "mouth-to-mouth".tw,kf. (713)
22. "mouth-to-skin".tw,kf. (163)
23. "Face to face".tw,kf. (45810)
24. Mass Gatherings/ (154)
25. mass gathering*.tw,kf. (1220)
26. festival*.tw,kf. (2133)
27. pride event*.tw,kf. (51)
28. exp Sexual Behavior/ (129446)
29. Sexually Transmitted Diseases/ or Sexually Transmitted Diseases, Viral/ (29686)
30. (Sexual* adj3 (interact* or activit* or behavio?r* or intercourse or transmi*)).tw,kf. (97840)
31. ((oral* or anal* or penetrat* or insert* or vaginal* or unprotected or condomless) adj3 sex*).tw,kf. (35543)
32. (nonsexual* adj3 (interact* or activit* or behavio?r* or transmi*)).tw,kf. (201)
33. (Intimate adj3 (interact* or activit* or behavio?r* or transmi*)).tw,kf. (1451)
34. (Person* adj3 (interact* or activit* or behavio?r* or transmi*)).tw,kf. (22345)
35. (direct* adj3 (interact* or activit* or behavio?r* or transmi*)).tw,kf. (87653)
36. (human* adj3 (interact* or activit* or behavio?r* or transmi*)).tw,kf. (133971)
37. (People* adj3 (interact* or activit* or behavio?r* or transmi*)).tw,kf. (15610)
38. kissing.tw,kf. (3205)
39. touch*.tw,kf. (45289)
40. (transmi* adj5 (skin or dermal* or dermis or epiderm* or genital* or lesion*)).tw,kf. (3471)
41. exp Body Fluids/ (347966).
42. exp Bodily Secretions/ (288070)
43. ((body or bodily) adj fluid*).tw,kf. (30273)
44. ((body or bodily) adj secretion*).tw,kf. (408)
45. Saliva*.tw,kf. (126280)
46. Mucus.tw,kf. (32610)
47. blood*.tw,kf. (2357256)
48. (Vaginal* adj (fluid* or secret* or discharge*)).tw,kf. (7139)
49. (penile adj (fluid* or secret* or discharge* or ejaculat*)).tw,kf. (57)
50. (ejaculate* adj fluid*).tw,kf. (18)
51. Semen.tw,kf. (38324)
52. seminal fluid*.tw,kf. (3121)
53. urine.tw,kf. (280658)
54. (f?eces or f?ecal*).tw,kf. (173918)
55. Air Microbiology/ (8471)
56. "Respiratory Aerosols and Droplets"/ (671)
57. aerosol*.tw,kf. (59763)
58. airborne.tw,kf. (28258)
59. Air* particle*.tw,kf. (3226)

60. droplet*.tw,kf. (68592)
61. exhalation*.tw,kf. (3740)
62. exhaled.tw,kf. (14056)
63. inhalation*.tw,kf. (76061)
64. inhaled.tw,kf. (53909)
65. air sampl*.tw,kf. (8347)
66. (respiratory adj (route* or mode* or path* or method* or transmi*)).tw,kf. (9175)
67. (airway* adj (route* or mode* or path* or method* or transmi*)).tw,kf. (1715)
68. (air way* adj (route* or mode* or path* or method* or transmi*)).tw,kf. (0)
69. (breath* adj (route* or mode* or path* or method* or transmi*)).tw,kf. (1709)
70. Fomites/ (662)
71. fomite*.tw,kf. (1565)
72. (surfac* or object or objects or cloth* or fabric* or bedding or towel* or needle*).tw,kf. (1999011)
73. (bed* or towel* or cloth* or house* or home* or dish* or cutlery* or cup* or drink*).tw,kf. (1348184)
74. contamina*.tw,kf. (314093)
75. cohabit*.tw,kf. (7472)
76. (household* or living together).tw,kf. (120661)
77. (Infection adj2 source*).tw,kf. (11035)
78. ((Virus or viral*) adj5 (touch* or surface*)).tw,kf. (10865)
79. (household* or living together).tw,kf. (120661)
80. Carrier State/ (22638)
81. or/14-80 (7558367)
82. 13 and 81 (2510)

Embase (1974 to 30 August 2024)

1. monkeypox/ (4620)
2. monkeypox virus/ (2351
3. poxvirus infection/ (1435)
4. poxviridae/ (1156)
5. monkeypox.tw,kf. (4673)
6. monkey pox.tw,kf. (133)
7. mpox*.tw,kf. (1963)
8. monkeypoxvir*.tw,kf. (20)
9. hMPXV*.tw,kf. (36)
10. MPXV*.tw,kf. (969)
11. MPX*.tw,kf. (1867)
12. chimpanzeepox.tw,kf. (1)
13. chimpanzee pox.tw,kf. (0)
14. or/1-13 (9085)

15. (transmi* adj5 (route* or mode or modes or path or paths or pathway* or method* or way* or how or direct* or indirect* or primary or secondary or pattern* or vehicle* or window*)).tw,kf. (77519)
16. transmi*.ti,kf. (159740)
17. exp *disease transmission/ (43434)
18. "Person to person".tw,kf. (5441)
19. "human to human".tw,kf. (8534)
20. contact*.tw,kf. (612959)
21. "Skin to skin".tw,kf. (11652)
22. "mouth-to-mouth".tw,kf. (847)
23. "mouth-to-skin".tw,kf. (286)
24. "Face to face".tw,kf. (60997)
25. mass gathering/ (411)
26. mass gathering*.tw,kf. (1341)
27. festival*.tw,kf. (2504)
28. pride event*.tw,kf. (75)
29. exp sexual behavior/ (260695)
30. sexually transmitted disease/ or viral sexually transmitted disease/ (54366)
31. (Sexual* adj3 (interact* or activit* or behavio?r* or intercourse or transmi*)).tw,kf. (125378)
32. ((oral* or anal* or penetrat* or insert* or vaginal* or unprotected or condomless) adj3 sex*).tw,kf. (50183)
33. (nonsexual* adj3 (interact* or activit* or behavio?r* or transmi*)).tw,kf. (226)
34. (Intimate adj3 (interact* or activit* or behavio?r* or transmi*)).tw,kf. (1657)
35. (Person* adj3 (interact* or activit* or behavio?r* or transmi*)).tw,kf. (28220)
36. (direct* adj3 (interact* or activit* or behavio?r* or transmi*)).tw,kf. (103316)
37. (human* adj3 (interact* or activit* or behavio?r* or transmi*)).tw,kf. (152554)
38. (People* adj3 (interact* or activit* or behavio?r* or transmi*)).tw,kf. (19299)
39. kissing.tw,kf. (4971)
40. touch*.tw,kf. (59855)
41. (transmi* adj5 (skin or dermal* or dermis or epiderm* or genital* or lesion*)).tw,kf. (4333)
42. exp "body fluids and secretions"/ (3409442)
43. ((body or bodily) adj fluid*).tw,kf. (33828)
44. ((body or bodily) adj secretion*).tw,kf. (437)
45. Saliva*.tw,kf. (147605)
46. Mucus.tw,kf. (42598)
47. blood*.tw,kf. (3196124)
48. (Vaginal* adj (fluid* or secret* or discharge*)).tw,kf. (9561)
49. (penile adj (fluid* or secret* or discharge* or ejaculat*)).tw,kf. (103)
50. (ejaculate* adj fluid*).tw,kf. (13)
51. Semen.tw,kf. (49191)
52. seminal fluid*.tw,kf. (3754)
53. urine.tw,kf. (374066)
54. (f?eces or f?ecal*).tw,kf. (219261)
55. environmental microbiology/ (992)

56. aerosol/ (67329)
57. exp airborne transmission/ (1705)
58. exp indirect contact transmission/ (2432)
59. aerosol*.tw,kf. (80255)
60. airborne.tw,kf. (35166)
61. Air* particle*.tw,kf. (4198)
62. droplet*.tw,kf. (78354)
63. exhalation*.tw,kf. (5542)
64. exhaled.tw,kf. (20827)
65. inhalation*.tw,kf. (99258)
66. inhaled.tw,kf. (81155)
67. air sampl*.tw,kf. (11217)
68. (respiratory adj (route* or mode* or path* or method* or transmi*)).tw,kf. (11802)
69. (airway* adj (route* or mode* or path* or method* or transmi*)).tw,kf. (3587)
70. (air way* adj (route* or mode* or path* or method* or transmi*)).tw,kf. (0)
71. (breath* adj (route* or mode* or path* or method* or transmi*)).tw,kf. (2442)
72. fomite/ (890)
73. fomite transmission/ (127)
74. fomite*.tw,kf. (1793)
75. (surfac* or object or objects or cloth* or fabric* or bedding or towel* or needle*).tw,kf. (2219988)
76. (bed* or towel* or cloth* or house* or home* or dish* or cutlery* or cup* or drink*).tw,kf. (1803308)
77. contamina*.tw,kf. (367250)
78. cohabit*.tw,kf. (8365)
79. (household* or living together).tw,kf. (138554)
80. (Infection adj2 source*).tw,kf. (14685)
81. ((Virus or viral*) adj5 (touch* or surface*)).tw,kf. (12258)
82. (household* or living together).tw,kf. (138554)
83. exp disease carrier/ (55638)
84. or/15-83 (10909054)
85. 14 and 84 (3926)
86. limit 85 to (conference abstract or editorial or letter) (1022)
87. 85 not 86 (2904)

Web of Science Preprint Citation Index

Date of search: 02/09/2024

TS=(monkeypox) OR TS=("monkey pox") OR TS=(mpox*) OR TS=(monkeypoxvir*) OR TS=(hMPXV*) OR TS=(MPXV*) OR TS=(MPX*) OR TS=(chimpanzeepox) OR TS=("chimpanzee pox")

And:

TS=((transmi* NEAR/4 (route* or mode or modes or path or paths or pathway* or method* or way* or how or direct* or indirect* or primary or secondary or pattern* or vehicle* or window*))) OR TI=(transmi*) OR KP=(transmi*) OR TS=("Person to person") OR TS=("human to human") OR TS=(contact*) OR TS=("Skin to skin") OR TS=("mouth-to-mouth") OR TS=("mouth-to-skin") OR TS=("Face to face") OR TS=("mass gathering*") OR TS=(festival*) OR TS=("pride event*") OR TS=((Sexual* NEAR/2 (interact* or activit* or behavio$r* or intercourse or transmi*))) OR TS=(((oral* or anal* or penetrat* or insert* or vaginal* or unprotected or condomless) NEAR/2 sex*) OR TS=((nonsexual* NEAR/2 (interact* or activit* or behavio$r* or transmi*))) OR TS=((Intimate NEAR/2 (interact* or activit* or behavio$r* or transmi*))) OR TS=((Person* NEAR/2 (interact* or activit* or behavio$r* or transmi*))) OR TS=((direct* NEAR/2 (interact* or activit* or behavio$r* or transmi*))) OR TS=((human* NEAR/2 (interact* or activit* or behavio$r* or transmi*))) OR TS=((People* NEAR/2 (interact* or activit* or behavio$r* or transmi*))) OR TS=(kissing) OR TS=(touch*) OR TS=((transmi* NEAR/4 (skin or dermal* or dermis or epiderm* or genital* or lesion*))) OR TS=(((body or bodily) NEAR/0 fluid*)) OR TS=(((body or bodily) NEAR/0 secretion*)) OR TS=(Saliva*) OR TS=(Mucus) OR TS=(blood*) OR TS=((Vaginal* NEAR/0 (fluid* or secret* or discharge*))) OR TS=((penile NEAR/0 (fluid* or secret* or discharge* or ejaculat*))) OR TS=((ejaculate* NEAR/0 fluid*)) OR TS=(Semen) OR TS=(“seminal fluid*”) OR TS=(urine) OR TS=((f$eces or f$ecal*)) OR TS=(aerosol*) OR TS=(airborne) OR TS=("Air* particle*") OR TS=(droplet*) OR TS=(exhalation*) OR TS=(exhaled) OR TS=(inhalation*) OR TS=(inhaled) OR TS=("air sampl*") OR TS=((respiratory NEAR/0 (route* or mode* or path* or method* or transmi*))) OR TS=((airway* NEAR/0 (route* or mode* or path* or method* or transmi*))) OR TS=(("air way*" NEAR/0 (route* or mode* or path* or method* or transmi*))) OR TS=((breath* NEAR/0 (route* or mode* or path* or method* or transmi*))) OR TS=(fomite*) OR TS=((surfac* or object or objects or cloth* or fabric* or bedding or towel* or needle*)) OR TS=((bed* or towel* or cloth* or house* or home* or dish* or cutlery* or cup* or drink*)) OR TS=(contamina*) OR TS=(cohabit*) OR TS=((household* or "living together")) OR TS=((Infection NEAR/1 source*)) OR TS=(((Virus or viral*) NEAR/4 (touch* or surface*))) OR TS=((household* or "living together"))

127 results

Protocol deviations

There has been one protocol deviation:

The inclusion criteria for exposure was amended to include unspecified clade as follows:

“Laboratory-confirmed infection with any clade of Mpox (Clade Ia, Ib, IIa, IIb **or unspecified clade**)

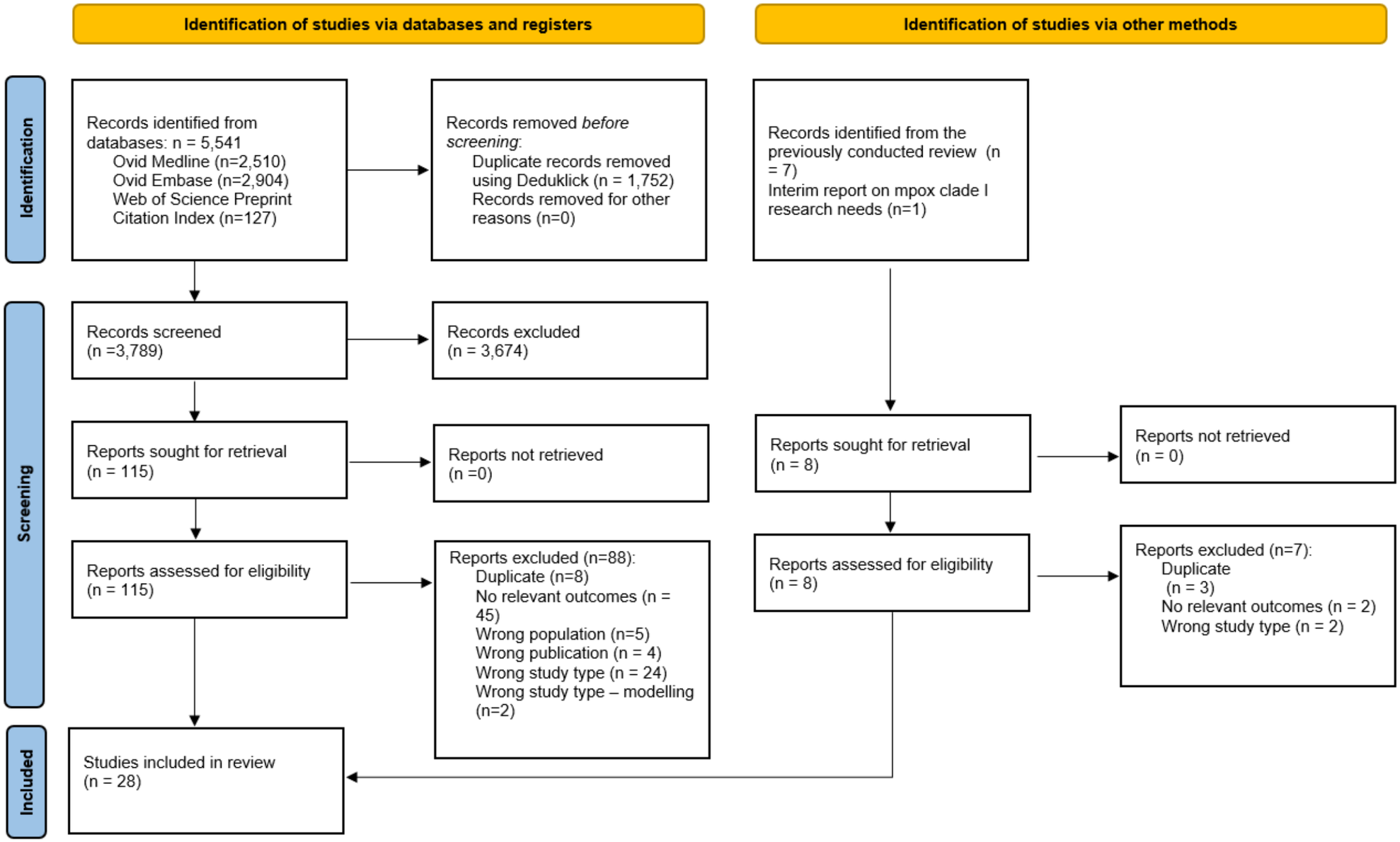
Or:

Clinically-suspected or laboratory-confirmed infection with mpox (Clade Ia, Ib, IIa, IIb, or unspecified) in Clade 1 outbreak countries (DRC, Republic of Congo, Central African Republic, Burundi, Rwanda, Uganda, Kenya, Cameroon, Gabon) since 1 January 2023”

This is to ensure that all information that may inform routes of transmission of mpox was included in this review.

Annexe B. Study selection flowchart

Figure B.1 PRISMA diagram



Text version of Figure B.1 PRISMA diagram

A PRISMA diagram showing the flow of studies through this review, ultimately including 28 studies.

From identification of studies via databases and registers, n = 5,541 records identified from databases:

- Ovid Medline (n = 2,510)
- Ovid Embase (n = 2,904)
- Web of Science Preprint Citation Index (n = 127)

From these, records removed before screening:

- duplicate records removed using Deduklick (n = 1,752)
- records removed for other reasons (n = 0)

n = 3,789 records screened, of which n = 3,674 were excluded, leaving n = 115 papers sought for retrieval, of which n = 0 were not retrieved.

n = 8 additional studies were identified from 2 relevant reviews. Seven additional studies was identified from a previous UKHSA mpox review on transmission (29) and one from a report on mpox clade I clinical presentation and severity of disease (31). Of which n = 8 were sought for retrieval, and n=1 was included.

Of the n = 123 papers assessed for eligibility, n=95 reports were excluded:

- duplicate (n = 11)
- no relevant outcomes (n = 47)
- wrong population (n = 5)
- wrong publication (n = 4)
- wrong study type (n = 26)
- wrong study type – modelling (n = 2)

n=28 papers included in the review.

Annexe C. Excluded full texts

Duplicate (11 studies)

Brosius and others. 'Pre- and asymptomatic viral shedding in high-risk contacts of monkeypox cases: A prospective cohort study' medRxiv. 2022: volume 27

Catala. 'Monkeypox outbreak in Spain: clinical and epidemiological findings in a prospective cross-sectional study of 185 cases' Br J Dermatol 2022: volume 187, issue 5, pages 765 to 772

Gould and others. 'Air and surface sampling for monkeypox virus in UK hospitals' medRxiv. 2022: volume 21

Marimuthu and others. 'Viable monkeypox virus in the environment of a patient room' medRxiv. 2022: volume 17

Snyder and others. 'Sexual exposures associated with mpox infection: California, November 2022 to June 2023' medRxiv. 2023: volume 9

Sypsa and others. 'Transmission potential of human monkeypox in mass gatherings' medRxiv. 2022: volume 21

Tarin-Vicente. 'Clinical presentation and virological assessment of confirmed human monkeypox virus cases in Spain: a prospective observational cohort study' Lancet 2022: volume 400, issue 10353, pages 661 to 669

van Ewijk and others. 'Monkeypox outbreak in the Netherlands in 2022: public health response, epidemiological and clinical characteristics of the first 1000 cases and protection of the first-generation smallpox vaccine' medRxiv 2022: volume 21

Vivancos. 'Community transmission of monkeypox in the United Kingdom, April to May 2022' Euro Surveill 2022: volume 27, issue 22

Yinda and others. 'Stability of mpox (monkeypox) virus in bodily fluids and wastewater' bioRxiv. 2023: volume 9

Minhaj and others. 'Monkeypox outbreak: 9 states, May 2022: weekly/June 10, 2022' American Journal of Transplantation 2022: volume 22, pages 2,104 to 2,110

No relevant outcomes (47 studies)

Blackburn and others. 'Epidemiologic and clinical features of mpox in transgender and gender-diverse adults: United States, May to November 2022' MMWR - Morbidity and Mortality Weekly Report 2022: volume 71, issue 5152, pages 1,605 to 1,609

Brosnan and others. 'Epidemiologic characteristics of mpox among people experiencing homelessness, Los Angeles County, California, USA, 2022' Emerging Infectious Diseases 2023: volume 29, issue 6, pages 1,109 to 1,116

Candela and others. 'Mpox DNA clearance in semen over 6-month follow-up' Journal of Medical Virology 2023: volume 95, issue 12, article e29259

Catala and others. 'Monkeypox outbreak in Spain: clinical and epidemiological findings in a prospective cross-sectional study of 185 cases' British Journal of Dermatology 2022: volume 187, issue 5, pages 765 to 772

Chin and others. 'Clinical presentation, viral shedding, and neutralizing antibody responses of mpox cases in South Korea: Single center experience' Journal of Clinical Virology 2024: volume 173, page 105,692

Contag and others. 'Prevalence of mpox (Monkeypox) in patients undergoing STI screening in northern California, April to September 2022' Journal of Clinical Virology 2023: volume 164, page 105493

Coppens and others. 'Alternative sampling specimens for the molecular detection of mpox (formerly monkeypox) virus' Journal of Clinical Virology 2023: volume 159, page 105,372

Damhorst and others. 'Multisite mpox infection and viral dynamics among persons with HIV in metro Atlanta' Journal of Infectious Diseases 2024: volume 229, pages S213 to S218

de Perio and others. 'Evaluation of mpox exposures and outcomes in workplaces, 6 jurisdictions, June 1 to August 31, 2022' Public Health Reports 2024: page 333549241245655

de Vries and others. 'Mpox outbreak among men who have sex with men in Amsterdam and Rotterdam, the Netherlands: no evidence for undetected transmission prior to May 2022, a retrospective study' Euro Surveillance: Bulletin Europeen sur les Maladies Transmissibles = European Communicable Disease Bulletin 2023: volume 28, issue 17, page 4

Du and others. 'The prevalence of mpox and its association with sexual behavior among Chinese men who have sex with men in early August 2023' Journal of Medical Virology 2023: volume 95, issue 12, article e29320

Edouard and others. 'Incidental diagnosis of mpox virus infection in patients undergoing sexually transmitted infection screening-findings from a study in France' International Journal of Infectious Diseases 2024: volume 143, page 107,009

Eser-Karlidag and others. 'Features of mpox infection: The analysis of the data submitted to the ID-IRI network' New Microbes and New Infections 2023: volume 53, page 101,154

Formenty and others. 'Human monkeypox outbreak caused by novel virus belonging to Congo Basin clade, Sudan, 2005' Emerging Infectious Diseases 2010: volume 16, issue 10, pages 1,539 to 1,545

Fu and others. 'Epidemiological characteristics, clinical manifestations, and mental health status of human mpox cases: a multicenter cross-sectional study in China' Journal of Medical Virology 2023: volume 95, issue 10, article e29198

Girometti. 'Demographic and clinical characteristics of confirmed human monkeypox virus cases in individuals attending a sexual health centre in London, UK: an observational analysis' Lancet Infect Dis 2022: volume 22, issue 9, pages 1,321 to 1,328

Grau Echevarria and others. 'Clinical and demographic features of 49 patients with human monkeypox virus-confirmed infection in a tertiary care center in Valencia, Spain: a descriptive study' Sexually Transmitted Diseases 2023: volume 50, issue 2, pages 66 to 73

Guo and others. 'Profiling of viral load, antibody and inflammatory response of people with monkeypox during hospitalization: a prospective longitudinal cohort study in China' EBioMedicine 2024: volume 106, article 105254

Huhn and others. 'Clinical characteristics of human monkeypox, and risk factors for severe disease' Clinical Infectious Diseases 2005: volume 41, pages 1,742 to 1,751

Ianache and others. 'Mpox across countries from Central and Eastern Europe - 2022 outbreak' Travel Medicine and Infectious Disease 2024: volume 59, page 102719

Kava and others. 'Epidemiologic features of the monkeypox outbreak and the public health response: United States, May 17 to October 6, 2022' MMWR - Morbidity and Mortality Weekly Report 2022: volume 71, issue 45, pages 1,449 to 1,456

Kroger and others. 'Mpox outbreak 2022: an overview of all cases reported to the Cologne Health Department' Infection 2023: volume 51, issue 5, pages 1,369 to 1,381

Lim and others. 'Correlation between monkeypox viral load and infectious virus in clinical specimens' Journal of Clinical Virology 2023: volume 161, page 105421

Lim and others. 'Clinical features of mpox patients in Korea: a multicenter retrospective study' Journal of Korean Medical Science 2024: volume 39, issue 4, page e19

Martinez de Victoria-Carazo and others. 'Mpox infection and sexually transmitted infections: a cross-sectional study from a secondary hospital in the May to September 2022 international outbreak' AIDS Research and Human Retroviruses 2023: volume 39, issue 11, pages 604 to 609

Miura and others. 'Time scales of human monkeypox transmission in the Netherlands' medRxiv. 2022: volume 4

Miura and others. 'Estimated incubation period for monkeypox cases confirmed in the Netherlands, May 2022' Euro Surveillance: Bulletin Europeen sur les Maladies Transmissibles = European Communicable Disease Bulletin 2022: volume 27, issue 24, page 6

Moraes-Cardoso and others. 'Immune responses associated with mpox viral clearance in men with and without HIV in Spain: a multisite, observational, prospective cohort study' The Lancet. Microbe 2024: volume 5, issue 8, page 100859

Moschese and others. 'Isolation of viable monkeypox virus from anal and urethral swabs, Italy, May to July 2022' Euro Surveillance: Bulletin Europeen sur les Maladies Transmissibles = European Communicable Disease Bulletin 2022: volume 27, issue 36, page 9

Nolen and others. 'Extended human-to-human transmission during a monkeypox outbreak in the Democratic Republic of the Congo' Emerging Infectious Diseases 2016: volume 22, issue 6, pages 1,014 to 1,021

Nolen and others. 'Introduction of monkeypox into a community and household: risk factors and zoonotic reservoirs in the Democratic Republic of the Congo' American Journal of Tropical Medicine and Hygiene 2015: volume 93, issue 2, pages 410 to 415

Norz and others. 'Clinical characteristics and comparison of longitudinal qPCR results from different specimen types in a cohort of ambulatory and hospitalized patients infected with monkeypox virus' Journal of Clinical Virology 2022: volume 155, page 105,254

Oakley and others. 'Mpox cases among cisgender women and pregnant persons: United States, May 11 to November 7, 2022' MMWR - Morbidity and Mortality Weekly Report 2023: volume 72, issue 1, pages 9 to 14

Orviz and others. 'Monkeypox outbreak in Madrid (Spain): clinical and virological aspects' Journal of Infection 2022: volume 85, issue 4, pages 412 to 417

Qiao and others. 'Global Mpox spread due to increased air travel' Geospatial Health 2024: volume 19, issue 1, page 11

Ramirez-Soto and others. 'Epidemiological and clinical characteristics of monkeypox among people with and without HIV in Peru: a national observational study' Journal of Infection and Public Health 2024: volume 17, issue 8, page 102,494

Rizzo and others. 'Concomitant diagnosis of sexually transmitted infections and human monkeypox in patients attending a sexual health clinic in Milan, Italy' Journal of Medical Virology 2023: volume 95, issue 1, article e28328

Siegenbeek van Heukelom and others. 'Characteristics of mpox positive, versus mpox negative, and mpox unsuspected clients from the Centre of Sexual Health, Public Health Service of Amsterdam, 20 May to 15 September 2022' Journal of the European Academy of Dermatology & Venereology 2023: volume 37, issue 9, pages 1,891 to 1,896

Ramirez-Olivencia and others. 'Clinical and epidemiological characteristics of the 2022 mpox outbreak in Spain (CEME-22 Study)' Open Forum Infectious Diseases 2024: volume 11, issue 3, page ofae105

Thomas and others. 'Notes from the field: transmission of mpox to nonsexual close contacts - 2 US jurisdictions, May 1 to July 31, 2022' MMWR - Morbidity and Mortality Weekly Report 2023: volume 72, issue 50, pages 1,351 to 1,352

Valentino and others. 'Clinical features and diagnostic challenges of mpox (monkeypox) outbreak in Malta: a retrospective cohort study' International Journal of Dermatology 2023: volume 62, issue 10, pages 1,266 to 1,271

Vanhamel and others. 'Understanding sexual transmission dynamics and transmission contexts of monkeypox virus: a mixed-methods study of the early outbreak in Belgium (May to June 2022)' Sexually Transmitted Infections 2023: volume 99, issue 5, pages 330 to 336

Vivancos and others. 'Community transmission of monkeypox in the United Kingdom, April to May 2022' Euro Surveillance: Bulletin Europeen sur les Maladies Transmissibles = European Communicable Disease Bulletin 2022: volume 27, issue 22, page 6

Vusirikala. 'Epidemiology of early monkeypox virus transmission in sexual networks of gay and bisexual men, England, 2022' Emerging Infectious Diseases 2022: volume 28, issue 10, pages 2,082 to 2,086

Yang and others. 'Clinical characteristics, viral dynamics, and antibody response of monkeypox virus infections among men with and without HIV infection in Guangzhou, China' Frontiers in Cellular and Infection Microbiology 2024: volume 14, page 1412753

Tarin-Vicente and others. 'Clinical presentation and virological assessment of confirmed human monkeypox virus cases in Spain: a prospective observational cohort study' Lancet 2022: volume 400, issue 10353, pages 661 to 669

Yinka-Ogunleye and others. 'Outbreak of human monkeypox in Nigeria in 2017 to 2018: a clinical and epidemiological report' The Lancet Infectious Diseases 2019: volume 19, issue 8, pages 872 to 879

Wrong population (5 studies)

Li and others. 'Stability of mpox virus on different commonly contacted surfaces' Journal of Medical Virology 2023: volume 95, issue 12, article e29296

Meister and others. 'Stability and Inactivation of Monkeypox Virus on Inanimate Surfaces' Journal of Infectious Diseases 2023: volume 228, issue 9, pages 1,227 to 1,230

Verreault and others. 'Susceptibility of monkeypox virus aerosol suspensions in a rotating chamber' Journal of Virological Methods 2013: volume 187, issue 2, pages 333 to 337

Yinda and others. 'Stability of Monkeypox Virus in Body Fluids and Wastewater' Emerging Infectious Diseases 2023: volume 29, issue 10, pages 2,065 to 2,072

Learned and others. 'Extended interhuman transmission of monkeypox in a hospital community in the Republic of the Congo, 2003' American Journal of Tropical Medicine and Hygiene 2005: volume 73, issue 2, pages 428 to 434

Wrong publication type (4 studies)

Anonymous. 'Human monkeypox: Kasai Oriental, Zaire, 1996 to 1997' Morbidity and Mortality Weekly Report 1997: pages 304 to 307

Raccagni and others. 'Monkeypox infection among men who have sex with men: PCR testing on seminal fluids' Journal of Infection 2022: volume 85, issue 5, pages 573 to 607

Thy and others. 'Breakthrough Infections after post-exposure vaccination against mpox' New England Journal of Medicine 2022: volume 387, issue 26, pages 2,477 to 2,479

York. 'The bodily distribution of monkeypox virus' Nature Reviews Microbiology 2022: volume 20, page 703

Wrong study type (26 studies)

Alegre and others. 'Otorhinolaryngological manifestations in monkeypox' Acta Otorrinolaringologica Espanola 2023: volume 74, issue 4, pages 263 to 267

Besombes and others. 'Intrafamily transmission of monkeypox virus, Central African Republic, 2018' Emerging Infectious Diseases 2019: volume 25, issue 8, pages 1,602 to 1,604

Cobos and others. 'Demographic, clinical and microbiological characteristics of the first 30 human monkeypox confirmed cases attended in a tertiary hospital in Madrid (Spain), during the May-June 2022 international outbreak' Revista Espanola de Quimioterapia 2023: volume 36, issue 2, pages 194 to 200

Gaspari and others. 'Monkeypox outbreak 2022: clinical and virological features of 30 patients at the Sexually Transmitted Diseases Centre of Sant' Orsola Hospital, Bologna, Northeastern Italy' Journal of Clinical Microbiology 2023: volume 61, issue 1, page e0136522

Heskin. 'Transmission of monkeypox virus through sexual contact: a novel route of infection' Journal of Infection 2022: volume 85, issue 3, pages 334 to 363

Hornuss and others. 'Transmission characteristics, replication patterns and clinical manifestations of human monkeypox virus-an in-depth analysis of 4 cases from Germany' Clinical Microbiology and Infection 2023: volume 29, issue 1, pages 112.e115 to 112.e119

Jia and others. 'Cases of monkeypox show highly-overlapping co-infection with HIV and syphilis' Frontiers in Public Health 2023: volume 11, page 127,6821

Kibungu and others. 'Clade I-associated Mpox cases associated with sexual contact, the Democratic Republic of the Congo' Emerging Infectious Diseases 2024: volume 30, issue 1, pages 172 to 176

Kowalski and others. 'Study of the first clinical cases on monkeypox in Poland' Przeglad Epidemiologiczny 2022: volume 76, issue 2, pages 168 to 183

Marimuthu and others. 'Viable mpox virus in the environment of a patient room' International Journal of Infectious Diseases 2023: volume 131, pages 40 to 45

Meo and others. 'Impact of traveling on transmission trends of human monkeypox disease: worldwide data based observational analysis' Frontiers in Public Health 2023: volume 11, page 1029215

Minhaj and others. 'Monkeypox outbreak: 9 states, May 2022' MMWR - Morbidity and Mortality Weekly Report 2022: volume 71, issue 23, pages 764 to 769

Nakoune and others. 'A nosocomial outbreak of human monkeypox in the Central African Republic' Open Forum Infectious Diseases 2017: volume 4, issue 4, page ofx168

Okoh and others. 'A virulent disease called monkeypox: a case report of countries in Africa where the disease is endemic' Indo Global Journal of Pharmaceutical Sciences 2022: volume 12, pages 237 to 244

Palich and others. 'Viral loads in clinical samples of men with monkeypox virus infection: a French case series' The Lancet Infectious Diseases 2023: volume 23, issue 1, pages 74 to 80

Peiro-Mestres and others. 'Frequent detection of monkeypox virus DNA in saliva, semen, and other clinical samples from 12 patients, Barcelona, Spain, May to June 2022' Euro Surveillance: Bulletin Europeen sur les Maladies Transmissibles = European Communicable Disease Bulletin 2022: volume 27, issue 28, page 7

Raymenants and others. 'Detection of mpox virus in ambient air in a sexual health clinic' Archives of Virology 2023: volume 168, issue 8, page 210

Relhan and others. 'Clinical presentation, viral kinetics, and management of human monkeypox cases from New Delhi, India 2022' Journal of Medical Virology 2023: volume 95, issue 1, article e28249

Sberna and others. 'Role of direct sexual contact in human transmission of monkeypox virus, Italy' Emerging Infectious Diseases 2024: volume 30, issue 9, pages 1,829 to 1,833

Schwartz. 'High rates of miscarriage and stillbirth among pregnant women with Clade I mpox (monkeypox) are confirmed during 2023 to 2024 DR Congo outbreak in South Kivu Province' Viruses 2024: volume 16, issue 7, page 13

Seang. 'Evidence of human-to-dog transmission of monkeypox virus' Lancet 2022: volume 400, issue 10353, pages 658 to 659

Tascini and others. 'Monkeypox virus transmission in tattoo parlor' New Microbiologica 2023: volume 46, issue 3, pages 315 to 316

Thornhill and others. 'Monkeypox virus infection in humans across 16 countries: April to June 2022' New England Journal of Medicine 2022: volume 387, issue 8, pages 679 to 691

Thornhill and others. 'Human monkeypox virus infection in women and non-binary individuals during the 2022 outbreaks: a global case series' Lancet 2022: volume 400, issue 10,367, pages 1,953 to 1,965

Yang and others. 'Clinical features and viral load variations of mpox: a retrospective study in Chongqing, China' BMC Infectious Diseases 2024: volume 24, issue 1, page 641

Le Vavasseur and others. 'Anal monkeypox disease: description of 65 cases' Diseases of the Colon and Rectum 2024: volume 67, issue 2, pages 280 to 285

Wrong study type: modelling (2 studies)

Pekar and others. 'Genomic epidemiology reveals 2022 mpox epidemic in New York City governed by heavy-tailed sexual contact networks' MedRxiv: the Preprint Server for Health Sciences 2024: volume 1, page 1

Sypsa and others. 'Transmission potential of human monkeypox in mass gatherings' Open Forum Infectious Diseases 2022: volume 9, issue 11, page ofac501

Annexe D. Data extraction tables

Abbreviations: CI: confidence interval, CD4: Cluster of Differentiation 4, Ct: cycle threshold, DNA: Deoxyribonucleic acid, HCW: healthcare worker, HIV: Human Immunodeficiency virus, IQR: interquartile range, mm: millimetre, mL: millilitre, MSM: men-who-have-sex-with-men, OR: Odds ratio, PCR: Polymerase Chain Reaction, PPE: personal protective equipment, PrEP: Pre-Exposure Prophylaxis, SD: standard deviation, STI: sexually transmitted infection, µL: microlitre, WHO: World Health Organisation

Table D.1. Summary of studies investigating route of transmission in mpox clade I

Study	Country, time period, study type	Population	Outcomes
Masirika and others, 2024 (preprint) (2)	Democratic Republic of the Congo, September 24 2023 to January 29 2024 Prospective cohort study	51 suspected or confirmed mpox cases were admitted to Kamituga hospital in Democratic Republic of the Congo. 37 cases laboratory-confirmed by PCR (26 females (50.1%), 25 males (49.9%), median age of females 20 years [IQR: 17 to 21 years], median age of males 23 years [IQR:18.5 to 30.5 years], 47 were heterosexual [22 females, 25 males], 0 were homosexual, 2 females were bisexual, 0 had known HIV status, one male had accompanying STI). 2 were confirmed negative for mpox by PCR (one female [50.0%], one male [50.0%]). 12 were pending laboratory confirmation. (4 females [33.3%], 8 males [66.6%]). No patients were vaccinated against mpox. Ethnicity not reported. Transmission links were investigated in 13 individuals. 54% male were male, no cases had known HIV status, age and vaccination history were not reported.	Transmission links between 13 individuals were investigated (54% male, age not reported). Index case (male, laboratory-confirmed) infected 6 individuals: 5 females were infected through heterosexual sexual contact, all of whom developed symptoms of mpox (4 of which were subsequently laboratory-confirmed cases), and one male (laboratory-confirmed) was infected through contact with medical items used to treat the index case. The index case had undefined contact with a further 25 unconfirmed people. Second link of transmission chain: Two cases (female, laboratory-confirmed) had heterosexual sexual contact with laboratory-confirmed male contacts of the index case (one contact each) One case (male, symptomatic but not laboratory-confirmed) had non-sexual contact with a laboratory-confirmed female contact of the index case. One case (male, symptomatic but not laboratory-confirmed) had undefined contact with a laboratory-confirmed male contact of the index case. Third link of transmission chain: One case (male, laboratory-confirmed) had heterosexual contact with a laboratory-confirmed female contact of a case infected by a case infected by the index case. One case (male, symptomatic but not laboratory-confirmed) had contact of a non-heterosexual nature with a contact of a case (male, symptomatic but not laboratory-confirmed) infected by a case infected by the index case. One case who developed symptoms of mpox had undefined contact with 95 unconfirmed people.
Besombes and others, 2023 (1)	Central African Republic, November 2021 to January 2022 Outbreak report	25 mpox cases (14 confirmed, 11 suspected). 14 confirmed cases (35.7% male, median age: 22 years [IQR: 5 to 27 years, range: 5 to 40 years])	16.4% reported sexual contact as the route of transmission, Other reported exposures included meal sharing (83.3%), hospital visits (70.8%), living in the same household (68.4%).

Study	Country, time period, study type	Population	Outcomes
		11 suspected cases (18.2% male, median age: 5 years [IQR: 2 to 20 years, range: 0 to 38 years]) One reported co-infection with HIV (CD4 counts and treatment status not reported). Sexual orientation, vaccination history and ethnicity of cases were not reported.	

Table D.2. Summary of studies investigating routes of transmission of mpox clade II or mixed clade

Study	Country or region, time period, study type	Population	Outcomes
Brosius and others, 2023 (4)	Belgium, June 24 to July 31 2022 Prospective cohort study	25 high risk contacts of 23 confirmed mpox clade IIb cases (median age 43 years (IQR: 36 to 51 years), 96% MSM, 72% of participants reported having sexual contact with an index case and 28% had non-sexual contact with index cases including household contacts and prolonged skin to skin contact). 20% were living with HIV . One individual was immunosuppressed (the study did not report if this individual was definitely or possibly infected). 5 participants received post exposure vaccination and 6 were vaccinated against smallpox during childhood. Sexual orientation and ethnicity were not reported for participants. Participants were defined as: Definitely infected: at least one sample with PCR Ct value of less than 34 Possibly infected: at least one sample with PCR Ct value of less than or equal to 34 to less than 37 Uninfected: all PCR Ct values more than 37. In asymptomatic individuals, 0 (0%) were definitely infected, 2 (40%) were possibly infected and 7 (58.3%) were uninfected.	Participants were followed up for a median of 16 days (IQR: 14 to 26 days) after their last high-risk contact. 18 cases (72%) reported having sexual contact with an index case (defined as receptive or insertive penetrative sex, or oral sex, irrespective of exposure time). Of these 8 out of 18 (66.7%) were definitely infected, while 4 out of 18 (22.2%) were possibly infected. 7 participants had non-sexual contact with an index case (5 household contacts of confirmed mpox cases, 2 prolonged (more than 15 minutes) skin-to-skin contact with confirmed mpox case). Of these, none were definitely infected, and one was possibly infected [A]. Infection status between sexual and non-sexual contacts: p = 0.03 Among the 8 definitely infected cases, 6 (75%) developed typical symptoms and 2 had atypical symptoms (only fever and only fatigue) Mpox virus was detected (at its earliest) 1 (n=3) to 4 (n=2) days before symptom onset in 5 definitely infected cases with typical mpox symptoms. Viral culture of 4 pre-symptomatically collected anorectal samples and 1 saliva sample was performed. Of these, mpox virus was detected in 3 cases. The fourth sample had insufficient volume for culture.

Study	Country or region, time period, study type	Population	Outcomes
		In symptomatic individuals, 8 (100%) of definitely infected showed symptoms. Of these, 5 (83.3%) were pre-symptomatic. Three (60%) of possibly infected individuals showed symptoms, and 5 (41.7%) uninfected showed symptoms.	
Dou and others, 2023 (9)	China, May 31 to June 21, 2023 Retrospective cohort study	37 confirmed clade IIb mpox cases (aged between 24 to 51 years, median age 30 years [IQR: 26.5 to 34.5 years], 16.2% aged over 40 years, all were men, 1 (2.7%) heterosexual, 2 (5.4%) bisexual, 34 (91.9%) homosexual. Nineteen (51.4%) were living with HIV of whom 9 had undetectable HIV levels, one had a HIV viral load of more than 20 copies per mL. 3 had CD4 counts between 300 and 500 per mm^3, 6 had counts of more than 500 per mm^3 and one individual did not provide it). Three individuals (8.1%) had previously received the smallpox vaccine. Ethnicity not reported. Of these 37 cases, 28 (75.7%) were diagnosed at outpatient appointments, 6 (16.2%) were traced through contact investigation, and 3 (8.1%) were identified through source tracing. 33 close contacts were identified for the 37 cases, defined as individuals who had close contact with mpox infected case, 4 days prior to the onset of symptoms. This included 8 regular male sexual partners, of whom 1 tested positive for mpox, 10 casual male sexual partners, of whom 5 tested positive for mpox. 6 family members, 6 roommates and 3 HCWs who were not wearing appropriate PPE.	32 MSM reported having engaged in sexual activity before contracting mpox. One man who identified as heterosexual reported sexual contact with a woman before contracting mpox. 33 close contacts were identified (18 regular or casual male sexual partners, 6 family members, 6 roommates and 3 HCWs). Of these, 6 (18.2%) tested positive for mpox, either upon detection, or by day 7 after their last exposure, including one regular and 5 casual sexual partners. Four of these were asymptomatic and 2 were symptomatic. No transmission to family members, roommates or HCWs was observed. No general contacts developed any symptoms related to mpox (but no information was provided as to whether they were tested for asymptomatic or pre-symptomatic infection, or how long they were followed up for).

Study	Country or region, time period, study type	Population	Outcomes
		Additionally, 39 general contacts were identified (HCWs, coworkers, social contacts, cohabitants, and individuals involved in handling case waste).	
Hens and others, 2023 (5)	Belgium, May 23 to September 20 2022 Prospective cohort study	155 men with confirmed mpox clade IIb infection (median age 39.0 years [IQR: 33 to 46 years], 34.2% living with HIV). CD4 count was higher than 500 cells per µL in 38 out of 43 (88.4%) patients with known CD4 count. One patient was reported to be immunosuppressed. 95.5% gay or bisexual MSM. Vaccination in mpox cases: • 16.1% childhood vaccination for smallpox • 1.3% vaccinated post-exposure • 0.6% vaccinated pre-exposure • 16.1% had unknown vaccination status Ethnicity not reported. 37 cases reported contact with suspected or confirmed mpox case.	30 out of 155 (19.4%) reported sexual contact specifically with a suspected or confirmed mpox case 3 weeks prior to symptom onset. 7 out of 155 (4.5%) reported household contact, skin to skin contact or non-touch contact within range of 1.5m with a suspected or confirmed mpox case 3 weeks prior to symptom onset. 118 out of 155 (76.1%) reported no contact at all with a suspected or confirmed mpox case 3 weeks prior to symptom onset. 145 out of 155 cases (93.5%) were sexually active, the types of sexual practice were: • anal-insertive: 92 out of 145 (63.4%) • anal-receptive: 95 out of 145 (65.5%) • oral: 69 out of 145 (47.6%) • vaginal: 8 out of 145 (5.5%) • unknown: 7 out of 145 (4.8%)
Hoxha and others, 2023 (10)	Global (WHO regions: Region of Americas, African Region, European Region, Eastern Mediterranean Region and Western pacific Region) January 1 2022 to May 22 2023 Cross-sectional study	1,118 mpox cases (clade I and II) in patients under 18 years old (58.5% males, 40.1% female, 1.4% unknown gender) • ethnicity: 61.8% from WHO region of the Americas, 30.3% from African Region, 7.5% from the European Region, less than 1% from Eastern Mediterranean Region • 3.3% MSM, 82.2% unknown sexual behaviour, 14.5% non-MSM • 1% were living with HIV, 68.5% unknown HIV status, 30.5% living without • HIV 0.6% immunosuppressed, 62.6% unknown immunosuppression status, 36.8% not immunosuppressed Case information was known for 1,102 cases.	Virus clade was assumed based on reporting country or sub-national area of circulating clades in 2022. 297 cases of mpox were reported to be clade I (98 cases in 0 to 4 years, 144 cases in 5 to 12 years, 55 cases in 13 to 17 years), and 805 cases were reported to be clade II (224 cases in 0 to 4 years, 208 in 5 to 12 years, 373 cases in 13 to 17 years). Data on transmission route were not separated by clade. [All data estimated from figures] Mpox cases by transmission type for cases 0 to 4 years old (data available for 28 cases, clade unknown): • sexual encounter: 0 (0.0%) • person to person: 11 (39.3%) • contact with contaminated material: 6 (21.4%) • healthcare associated infection: 1 (3.6%) • mother to child at pregnancy or birth: 1 (3.6%) • other: 9 (32.1%)

Study	Country or region, time period, study type	Population	Outcomes
		328 (29.3%) aged 0 to 4 years (51.2% males, 47.0% females, 1.8% unknown gender) • 0% MSM, 100% unknown sexual behaviour, 0% non-MSM • 0% living with HIV, 74.4% unknown HIV status, 25.6% living without HIV • 0.3% immunosuppressed, 35.4% unknown immunosuppression status, 64.3% not immunosuppressed 353 (31.6%) aged 5 to 12 years (51.6% males, 48.2% females, 0.3% unknown gender) • 0% MSM, 87.8% unknown sexual behaviour, 12.2% non-MSM • 0.6% were living with HIV, 72.8% unknown HIV status, 26.6% living without HIV • 0.6% immunosuppressed, 65.7% unknown immunosuppression status, 33.7% not immunosuppressed 437 (39.1%) aged 13 to 17 years (69.6% males, 28.4% females, 2.1% unknown gender) • 8.5% MSM, 64.3% unknown sexual behaviour, 27.2% non-MSM • 2.1% were living with HIV, 60.6% unknown HIV status, 37.3% living without HIV • 0.9% immunosuppressed, 58.8% unknown immunosuppression status, 40.3% not immunosuppressed Vaccination status not reported	Mpox cases by transmission type for cases 5 to 12 years old (data available for 25 cases, clade unknown) • sexual encounter: 0 (0.0%) • person to person: 10 (40.0%) • contact with contaminated material: 6 (24.0%) • healthcare associated infection: 0 (0.0%) • mother to child at pregnancy or birth: 0 (0.0%) • other: 9 (36.0%) Mpox cases by transmission type for cases 13 to 17 years old (data available for 64 cases, clade unknown) • sexual encounter: 34 (53.1%) • person to person: 12 (18.8%) • contact with contaminated material: 3 (4.7%) • healthcare associated infection: 2 (3.1%) • mother to child at pregnancy or birth: 0 (0.0%) • other: 13 (20.3%)
Inigo Martinez and others, 2022 (7)	Spain, 17 May to 22 June 2022 Cross-sectional study.	508 mpox clade IIb cases. 503 (99%) were men and 5 (1%) were women. Median age 35 years (IQR: 12 years, range: 18 to 67 years): • under 20 years: 4 (0.8%) • 20 to 29 years: 115 (22.6%)	45 clusters with 96 linked cases were identified, ranging from 2 to 4 cases per cluster. 19 transmission chains with 42 cases were identified made up of 20 primary cases, and 21 secondary cases. All were described as close contacts during sexual activities, 13 between household members and 8 with non-household members. One additional tertiary case identified.

Study	Country or region, time period, study type	Population	Outcomes
		• 30 to 39 years: 211 (41.5%) • 40 to 49 years: 129 (25.4%) • 50 to 59 years: 43 (8.5%) • 60 to 69 years: 6 (1.2%) 397 (93%) were MSM 225 (44.3%) living with HIV 56 (11%) on PrEP Ethnicity and vaccination history not reported	Additional secondary transmission was reported in household setting in 13 close household contacts (11 [84.6%] men, 2 [15.4%] women) but type of contact was undefined. 408 cases were reportedly unaware of or reported no contact with a known case of mpox.
Laurenson-Schafer and others, 2023 (3)	Global (WHO regions: African region, Region of the Americas, Eastern Mediterranean region, South East Asia region, Western Pacific region), January 1 2022 to 29 January 2023 Cross-sectional study	Data from 82,807 mpox (primarily clade IIb) cases taken from global surveillance data was analysed. 21,749 cases had data on mode of transmission, as reported to WHO (21,145 (97.2%) men, 582 (2.8%) female, 12 (less than 1%) other, 10 (less than 1%) unknown gender) WHO regions for where data was available (confirmed cases with case details provided) African region: 401 (245 males, 156 females) Region of the Americas: 56,638 (48,111 males, 2109 females, 2 other, 6,416 unknown gender) Eastern Mediterranean region: 57 (53 males, 4 females) European region: 25,542 (25,051 males, 429 females, 12 other, 50 unknown) South East Asia region: 38 (22 males, 16 females) Western Pacific region: 131 (78 male, 5 female, 48 unknown gender) Number of cases in each age group of those with mode of transmission data (% of all cases) 0 to 9 years: 46 (0.2%) 10 to 17 years: 85 (0.4%) 18 to 29 years: 6,106 (28.1%) 30 to 39 years: 9,013 (41.4%) 40 to 49 years: 4,741 (21.8%)	Total numbers by transmission type [B]: Contact with contaminated material: 303. Healthcare associated transmission: 94 Vertical transmission during pregnancy or birth: 1 Person-to-person transmission: 2,374 Transmission by sexual encounter: 14,941 Transmission by occupational exposure: 9 Parenteral transmission: 2 Other transmission: 4,012 The most reported mode of transmission was via sexual encounter (n = 14,941, 68.7%) Routes of transmission by age group: 0 to 9 years (n=46): Contaminated material associated transmission by age group: 9 cases (19.6%) Healthcare associated transmission: 1 case (2.2%) Vertical transmission during pregnancy or birth: 1 case (2.2%) Person-to-person transmission: 15 cases (32.6%) Transmission by sexual encounter: 1 case (2.2%) Transmission by occupational exposure: 0 cases (0%) Parenteral transmission: 0 cases (0%) Other transmission: 19 cases (41.3%) 10 to 17 years (n=85): Contaminated material associated transmission by age group: 5 cases (5.9%) Healthcare associated transmission: 2 cases (2.4%)

Study	Country or region, time period, study type	Population	Outcomes
		50 to 59 years: 1,409 (6.5%) 60 to 69 years: 297 (1.4%) 70 to 79 years: 39 (0.2%) 80 years or older: 6 (less than 1%) Unknown: 7 (less than 1%) Number of cases per sexual orientation of those with mode of transmission data (% of all cases): 13,773 (63.3%) MSM 3,355 (15.4%) other sexual behaviour 4,621 (21.2%) unknown sexual behaviour Of 35,329 cases with known HIV status, 16,961 (48%) were living with HIV. Most cases living with HIV were living in the Americas (12,997 [52.4%] of 24,816), 3,950 (37.8%) of 10,440 were living in the European region. In other regions less than 50 known HIV cases were reported in each of the other regions. No vaccination data was reported.	Vertical transmission during pregnancy or birth: 0 cases (0%) Person-to-person transmission: 13 cases (15.3%) Transmission by sexual encounter: 31 cases (36.5%) Transmission by occupational exposure: 0 cases (0%) Parenteral transmission: 0 cases (0%) Other transmission: 34 cases (40%) 18 to 29 years (n=6,106): Contaminated material associated transmission by age group: 97 cases (1.6%) Healthcare associated transmission: 32 cases (0.5%) Vertical transmission during pregnancy or birth: 0 cases (0%) Person-to-person transmission: 611 cases (10%) Transmission by sexual encounter: 3,859 cases (63.2%) Transmission by occupational exposure: 3 cases (less than 0.1%) Parenteral transmission: 1 case (less than 0.1%) Other transmission: 1,497 cases (24.5%) 30 to 39 years (n=9,013): Contaminated material associated transmission by age group: 122 cases (1.4%) Healthcare associated transmission: 30 cases (0.3%) Vertical transmission during pregnancy or birth: 0 cases (0%) Person-to-person transmission: 1,027 cases (11.4%) Transmission by sexual encounter: 6,183 cases (68.6%) Transmission by occupational exposure: 3 cases (less than 0.1%) Parenteral transmission: 1 case (less than 0.1%) Other transmission: 1,641 cases (18.2%) 40 to 49 years (n=4,741): Contaminated material associated transmission by age group: 49 cases (1%) Healthcare associated transmission: 22 cases (0.5%) Vertical transmission during pregnancy or birth: 0 cases (0%) Person-to-person transmission: 519 cases (10.9%) Transmission by sexual encounter: 3,452 cases (72.8%) Transmission by occupational exposure: 1 case (less than 0.1%) Parenteral transmission: 0 cases (0%) Other transmission: 697 cases (14.7%) 50 to 59 years (n=1,409): Contaminated material associated transmission by age group: 17 cases (1.2%) Healthcare associated transmission: 3 cases (0.2%)

Study	Country or region, time period, study type	Population	Outcomes
			Vertical transmission during pregnancy or birth: 0 cases (0%) Person-to-person transmission: 155 cases (11%) Transmission by sexual encounter: 1,130 cases (80.2%) Transmission by occupational exposure: 1 case (0.1%) Parenteral transmission: 0 cases (0%) Other transmission: 103 cases (7.4%) 60 to 69 years (n=297): Contaminated material associated transmission by age group: 2 cases (0.7%) Healthcare associated transmission: 1 case (0.3%) Vertical transmission during pregnancy or birth: 0 cases (0%) Person-to-person transmission: 21 cases (7.1%) Transmission by sexual encounter: 255 cases (85.9%) Transmission by occupational exposure: 1 case (0.3%) Parenteral transmission: 0 cases (0%) Other transmission: 17 cases (5.7%) 70 to 79 years (n=39): Contaminated material associated transmission by age group: 1 case (1.2%) Healthcare associated transmission: 2 cases (5.1%) Vertical transmission during pregnancy or birth: 0 cases (0%) Person-to-person transmission: 9 cases (23.1%) Transmission by sexual encounter: 23 cases (59%) Transmission by occupational exposure: 0 cases (0%) Parenteral transmission: 0 cases (0%) Other transmission: 4 cases (10.3%) 80 years or older (n=6): Contaminated material associated transmission by age group: 1 case (16.7%) Healthcare associated transmission: 1 case (16.7%) Vertical transmission during pregnancy or birth: 0 cases (0%) Person-to-person transmission: 1 case (16.7%) Transmission by sexual encounter: 3 cases (50%) Transmission by occupational exposure: 0 cases (0%) Parenteral transmission: 0 cases (0%) Other transmission: 0 cases (0%) Unknown (n=7): Contaminated material associated transmission by age group: 0 cases (0%) Healthcare associated transmission: 0 cases (0%)

Study	Country or region, time period, study type	Population	Outcomes
			Vertical transmission during pregnancy or birth: 0 cases (0%) Person-to-person transmission: 3 cases (42.9%) Transmission by sexual encounter: 4 cases (57.1%) Transmission by occupational exposure: 0 cases (0%) Parenteral transmission: 0 cases (0%) Other transmission: 0 cases (0%) Routes of transmission by gender: Female (n=582): Contaminated material associated transmission: 39 cases (6.7%) Healthcare associated transmission: 30 cases (5.2%) Vertical transmission during pregnancy or birth: 1 case (0.2%) Person-to-person transmission: 94 cases (16.2%) Transmission by sexual encounter: 232 cases (39.9%) Transmission by occupational exposure: 4 cases (0.7%) Parenteral transmission: 0 cases (0%) Other transmission: 182 cases (31.3%) Male (n=21,145): Contaminated material associated transmission: 262 cases (1.2%) Healthcare associated transmission: 64 cases (0.3%) Vertical transmission during pregnancy or birth: 0 cases (0%) Person-to-person transmission: 2,278 cases (10.8%) Transmission by sexual encounter: 14,691 cases (69.5%) Transmission by occupational exposure: 5 cases (less than 0.1%) Parenteral transmission: 2 cases (less than 0.1%) Other transmission: 3,830 cases (18.1%) Other gender (n=12): Contaminated material associated transmission: 1 case (8.3%) Healthcare associated transmission: 0 cases (0%) Vertical transmission during pregnancy or birth: 0 cases (0%) Person-to-person transmission: 1 case (8.3%) Transmission by sexual encounter: 10 cases (83.3%) Transmission by occupational exposure: 0 cases (0%) Parenteral transmission: 0 cases (0%) Other transmission: 0 cases (0%) Unknown gender (n=10): Contaminated material associated transmission: 1 case (10%) Healthcare associated transmission: 0 cases (0%)

Study	Country or region, time period, study type	Population	Outcomes
			Vertical transmission during pregnancy or birth: 0 cases (0%) Person-to-person transmission: 1 case (10%) Transmission by sexual encounter: 8 cases (80%) Transmission by occupational exposure: 0 cases (0%) Parenteral transmission: 0 cases (0%) Other transmission: 0 cases (0%) Routes of transmission by sexual orientation: MSM (n=13,773): Contaminated material associated transmission: 142 cases (1%) Healthcare associated transmission: 27 cases (0.2%) Vertical transmission during pregnancy or birth: 0 cases (0%) Person-to-person transmission: 1,294 cases (9.4%) Transmission by sexual encounter: 10,252 cases (74.4%) Transmission by occupational exposure: 0 cases (0%) Parenteral transmission: 0 cases (0%) Other transmission: 2,055 cases (14.9%) Other Sexual Orientation (n=3,355): Contaminated material associated transmission: 111 cases (3.3%) Healthcare associated transmission: 43 cases (1.3%) Vertical transmission during pregnancy or birth: 0 cases (0%) Person-to-person transmission: 264 cases (7.9%) Transmission by sexual encounter: 1,192 cases (35.5%) Transmission by occupational exposure: 6 cases (0.2%) Parenteral transmission: 0 cases (0%) Other transmission: 1,729 cases (51.5%) Unknown Sexual Orientation (n=4,621) Contaminated material associated transmission: 50 cases (1.1%) Healthcare associated transmission: 24 cases (0.5%) Vertical transmission during pregnancy or birth: 1 case (less than 0.1%) Person-to-person transmission: 816 cases (17.7%) Transmission by sexual encounter: 3,497 cases (74.7%) Transmission by occupational exposure: 3 cases (0.1%) Parenteral transmission: 2 cases (less than 0.1%) Other transmission: 228 cases (4.9%) Sexual transmission by region: European region: 9,133 of 9,711 likely transmission events (94.0%)

Study	Country or region, time period, study type	Population	Outcomes
			South-East Asian region: 14 of 15 likely transmission events (93.3%) Western Pacific region: 8 of 10 likely transmission events (80.0%) Region of the Americas: 5,780 of 12,000 likely transmission events (48.2%) Eastern Mediterranean region: 6 of 13 likely transmission events (46.0%) Health workers represented 1,221 (5.3%) of 28,549 cases where health worker status was reported. 32 (10.1%) of 316 reported occupational exposure: 27 while providing health care to patients and 5 in a clinical laboratory. The remaining health workers reported having been infected mostly through sexual contact. [B] Transmission routes are not mutually exclusive, as country officials could report more than one mode of transmission.
Leonard and others, 2023 (8)	US, May 4 to August 17 2023 Outbreak report	56 confirmed clade IIb mpox cases, reported to Los Angeles County Department of Public Health (median age 35 years [IQR: 26 to 42 years]) 21 (38%) were non-Hispanic White (White) men, 18 (32%) were Hispanic or Latino (Hispanic), 13 (23%) were non-Hispanic Black or African American (Black), and 4 (7%) identified as another race 56% male 45 (80%) identified as gay or bisexual 17 (30%) were living with HIV. 3 patients with HIV had CD4 count below 350 cells per mm^3, 14 CD4 above 350, and 6 were considered not virally suppressed. 32 (57%) were unvaccinated, 8 (14%) were partially vaccinated, 16 (29%) were fully vaccinated against mpox.	55 of 56 lab-confirmed cases were interviewed. Of 55 lab-confirmed cases, 7 (13%) reported undefined contact with someone with mpox symptoms in the 3 weeks before symptom onset. 48 of 55 cases (87%) reported sexual contact in 3 weeks preceding symptom onset. Two pairs of patients (positive for mpox) disclosed sexual contact with another patient (confirmed positive for mpox) in 3 weeks preceding symptom onset. 1 case (2.2%) associated with travel to China.
Mazzotta and others, 2024 (6)	Italy, May to September 2023 Retrospective cohort study	541 mpox clade IIb cases at 15 Italian health centres (median age 38 years [IQR: 33 to 44 years], 404 (74.68%) were Caucasian, 4 (0.74%) were women, 512 (94.64%) were omo-bisexual [gay or bisexual])	502 (92.79%) participants reported sexual transmission. 39 participants reported non-sexual contact.

Study	Country or region, time period, study type	Population	Outcomes
		235 (43.44%) were living with HIV, 22 (4.07%) had CD4 count less than 350 cells per µL 61 (11.28%) had previous smallpox vaccination	

Table D.3. Summary of studies investigating routes of transmission of mpox clade not reported

Study	Country or region, time period, study type	Population	Outcomes
Angelo and others, 2023 (11)	Global (Argentina, Belgium, Canada, Denmark, France, Germany, Israel, Netherlands, Portugal, Romania, South Africa, Spain, Sweden, UK, USA), May 1 to July 1 2022 Cross-sectional study.	226 cases of mpox (median age 37 years (range: 18 to 68 years [IQR: 32 to 43 years]) from18 Sentinel sites across 15 countries (% of patients): Spain (35%), Canada (29%), Germany (7%), France (7%), Belgium (6%), Netherlands (4%), Portugal (3%), Sweden (3%), Romania (2%), USA (1%), Israel (1%), South Africa (1%), UK (1%), Denmark (1%), Argentina (1%). All cases were male. Of 211 patients with available information, 207 (98%) were MSM, 1 (0.6%) reported having both male and female partners, and 3 (1.4%) had only female partners. Of 209 patients with available data, 92 (44%) living with HIV. Median CD4 count was 713 cells per mm^3 (range: 36 to 1,659 cells per mm^3, [IQR: 500 to 885 cells per mm^3]). 76 (92%) had undetectable viral load. One patient had CD4 count of less than 200 cells per mm^3. 4 (2%) of 209 patients were immunocompromised by a condition other than HIV. 16 (9%) of 182 patients with available information had history of smallpox vaccination. Eight patients were health-care workers, all were MSM.	78 patients (40% of 195 with available information) reported contact with a person suspected or confirmed mpox. The type of contact was reported for 71 patients: Sexual or close intimate contact: 70 out of 71 (99%) Household contact: 8 out of 71 (11%). All patients with household contact also had sexual contact within their household. Face-to-face contact not in household: 2 (3%) All patients with face-to-face contact outside their household all also had sexual contact within their household. Other: 3 (4%). All patients with other forms of contact reported being in a couple with a confirmed case. Type of sexual or close intimate contact was reported for 48 patients: Penile-anal: 35 out of 48 (73%) Oral-penile: 32 out of 48 (67%) Oral-anal: 28 out of 48 (58%) Kissing, with or without additional sexual intimacy: 16 out of 48 (33%) Cuddling, with or without additional sexual intimacy: 15 out of 48 (31%) Mutual masturbation: 5 out of 48 (10%) Sharing sex toys: 2 out of 48 (4%) Nipple trauma: one out of 48 (2%) Fisting: 2 out of 48 (4%) Oral-vaginal: 0 out of 48 (0%) Penile-vaginal: 0 out of 48 (0%) Anatomical sites of exposure were reported for 46 patients: Penis: 39 out of 46 (85%) Pharynx: 32 out of 46 (70%) Rectum: 31 out of 46 (67%)

Study	Country or region, time period, study type	Population	Outcomes
			Face: 2 out of 46 (4%) For the 8 HCWs included in this cohort there was no evidence of nosocomial transmission.
Caria and others, 2022 (12)	Portugal, May 5 to July 26 2022 Cross-sectional study	41 confirmed mpox cases, with a median age of 37 years (range: 22 to 58 years). The patients' nationalities were: 18 (43.9%) Brazilians, 15 (36.6%) Portuguese, 2 (4.9%) French, 2 (4.9%) Colombians, and one (2.4%) each from Spain, Peru, Cape Verde, and Lebanon. 40 (97.6%) were male, and one (2.4%) was female. 38 (92.7%) MSM, 2 (4.9%) were men who have sex with both women and men, and one (2.4%) was a woman who has sex with women. 25 (61%) of the 41 patients were living with HIV (100% male, median age 37 years [IQR: 12 years]). Median CD4 cell count before mpox diagnosis was 702 cells per mm^3 (mean: 776 [SD: 377.5], range 244 to 1,728). All were on antiretroviral therapy. 3 had detectable viral load above 50 copies per mL. None of the 3 patients qualified for virological failure, as they had been on antiretroviral therapy for under 6 months and demonstrated a steady reduction in viral load. Most patients living with HIV were using a 3-drug regimen, with integrase inhibitors being the most common third drug. The mean age of living with HIV was statistically significantly higher than those who were living without HIV (p=0.013) 12 (75%) of 16 cases living without HIV took PrEP. 3 (7.3%) cases reported or registered history of smallpox vaccination. All were living with HIV, and they were 44, 53, and 54 years old, respectively.	16 (39%) cases reported sexual contact with mpox confirmed cases, of which one case also reported needle sharing for drug injection. 12 (48%) of the 25 people living with HIV reported sexual contact with a confirmed mpox case. The case who reported needle sharing and sexual contact with a confirmed case was also living with HIV.
Cassir and others, 2022 (13)	France, June 4 to August 31 2022 Retrospective cohort study	136 confirmed mpox cases. Median age 36 years (IQR: 30 to 42 years). 133 (97.8%) were men, 3 (2.2%) were women. 125 (92%) were MSM, 5 (4%) were heterosexual (2 men and 3 women), 6 did not disclose sexual orientation.	21 (15.4%) reported sexual contact with mpox confirmed case, including one woman who declared her regular sexual partner was diagnosed with mpox.

Study	Country or region, time period, study type	Population	Outcomes
		21 (15.4%) of 136 cases were HIV-positive, of whom 5 (23.8%) had a CD4 cell count of less than 500 cells per mm^3, and 0 (0%) had a CD4 cell count of less than 200 CD4 per mm^3. 30 (24%) of 136 cases using PrEP. An accompanying diagnosis of another STI occurred for 19 (15%) of 136 patients, including 2 patients who had a new diagnosis of HIV infection. 15 (11%) reported previous smallpox vaccination. 7 (5.1%) during childhood, 6 (4.4%) post exposure and 2 (1.5%) pre-exposure Ethnicity not reported	
Fernandez Pardal and others, 2024 (14)	Argentina, July 1 to October 31 2022 Retrospective cohort study.	124 confirmed mpox cases. The median age was 31.5 years (IQR: 28 to 38 years). 123 (99.2%) were born male, one (0.8%) who identified as a transwoman, 1 (0.8%) case was born and identified as female. 107 (86.3%) were homosexual, 5 (4%) identified as heterosexual, 5 (4%) as bisexual and 5 (4%) did not report sexual orientation. 75 (60.5%) were living with HIV of whom 10 (13.3%) had a CD4 T lymphocyte count of less than 350 per mm^3, 50 (66.7%) had a CD4 T lymphocyte count of more than 350 per mm^3, 15 had unknown CD4 count. 64 (85.3%) were taking antiretroviral therapy. 6 (4.8%) took PrEP 15 (12.1%) received a smallpox vaccine during childhood. 0 took the mpox vaccination. Ethnicity not reported. Confirmed epidemiological link was defined as when a sexual partner, non-sexual contact living with the case or a non-sexual contact not living with the case had a confirmed diagnosis of mpox. Probable epidemiological link was defined as when a patient	32 (25.8%) out of 124 cases had a confirmed epidemiological link: 28 (87.5%) out of 32 cases referred to a sexual contact as the source 2 (6.2%) out of 32 referred to a non-sexual partner cohabitant as the source 2 (6.2%) out of 32 cases referred to a non-sexual contact outside the home environment as the source Among the 124 patients, 62 (50%) had a probable epidemiological link, and in 26 (20.9%) cases no epidemiological link was found. In 4 cases (3.2%) no information on epidemiological link was available.

Study	Country or region, time period, study type	Population	Outcomes
		had sexual contact during the previous 21 days, in an anatomical site coinciding with the site of the initial lesions. No link was defined as absence of the aforementioned criteria.	
Fleischauer and others, 2005 (20)	US, June 4 to June 20 2003 Prospective cohort study.	57 HCWs were exposed to 3 mpox confirmed cases. Median age was 39 years (range: 19 to 61 years), 42 (74%) female, and 15 (26%) male, 20 (35%) were nursing staff, 16 (28%) were technicians, 10 (18%) were physicians, 6 (10%) were emergency medical service staff, and 5 (9%) were admin staff. 31 (54%) reported smallpox vaccination. 4 of these were less than 6 months before exposure. 3 of these 4 recently vaccinated HCWs had also received vaccination in childhood. Ethnicity, HIV status, and sexual orientation not reported.	HCWs were evaluated for monkeypox virus infection by ELISA of paired acute- and convalescent-phase serum specimens for anti-orthopoxviral IgM and IgG reactivity. Exposure proximity: Exposed to same air: 52 (91%) of 57 exposed HCWs. Exposed to same room: 52 (91%) of 57 exposed HCWs. Skin-on-skin contact: 28 (49%) of 57 exposed HCWs. Touched patient belongings: 46 (81%) of 57 exposed HCWs. Median number of exposures per healthcare worker: 2 (range: one to 68) Median duration of exposures: 10 min (range: one to 75 mins) Unprotected exposure: 40 (70%) of 57 exposed HCWs. 17 (29%) reported consistently using gloves, a gown, and either a surgical mask or N95 respirator during all interactions with monkeypox patients. While 35 HCWs (61%) used gloves for every patient encounter, the use of gowns (33%), surgical masks (25%), and N95 respirators (19%) was less common. None of the HCWs reported symptoms consistent with mpox. One HCW, who had been recently vaccinated for smallpox, tested positive for anti-orthopoxvirus IgM in both acute and convalescent serum samples. This HCW had one prior exposure, where vital signs were checked, and a physical exam was conducted. Gloves were worn during the encounter, and no skin contact was recalled. No symptoms appeared within 21 days of exposure. Among 31 previously vaccinated HCWs who were exposed, 29 (94%) tested positive for anti-orthopoxvirus IgG antibodies. Of the 26 HCWs with no known vaccination history, 3 tested positive for IgG antibodies in both acute and convalescent serum samples, all of whom were born before 1970, during the routine smallpox vaccination era.

Study	Country or region, time period, study type	Population	Outcomes
Hennessee and others, 2022 (19)	US, May 24 to June 17 2022 Cross-sectional study	83 mpox cases (children and adolescents aged under 18 years). 38 (47%) were black, 28 (35%) are Hispanic or Latino, 10 (12%) were white, 2 (2%) were Asian, 1 (1%) were American Indian or Alaska native, 1 (1%) native Hawaiian or other, 1 (1%) were other, 2 (2%) were unknown ethnicity. 66 (80%) born as male, 16 (20%) born as female (1 transgender male), 1 unknown gender. Age groups: 0 to 4 years (n=16): 12 (75%) male, 4 (25%) female. 7 (44%) Black, 5 (31%) Hispanic or Latino, 3 (19%) white, 1 (6%) Native Hawaiian or other pacific islander. 5 to 12 years (n=12): 6 (50%) male, 6 (50%) female. 5 (42%) black, 5 (42%) Hispanic or Latino, 2 (17%) white. 13 to 17 years (n=55): 48 (89%) male, 6 (11%) female, 1 unknown. 26 (49%) black, 18 (34%) Hispanic or Latino, 5 (9%) white, 2 (4%) Asian, 1 (2%) American Indian or Alaska Native, 1 (2%) other, 2 (4%) unknown. Sexual orientation not reported, but the following sexual contact was reported in 34 cases (32 male, 1 female, 1 transgender male): 23 (72%) reported male-to-male sexual contact, 4 (13%) reported male-to-female sexual contact, 5 (16%) reported sexual contact with a person whose sex was not reported. A female adolescent reported recent sexual contact with a male. The transgender male reported recent sexual contact with a male adolescent. JYNNEOS vaccination was offered to close contacts in at least 4 situations, and in one instance more than 15 other students and staff members received JYNNEOS postexposure prophylaxis. HIV status not reported.	Overall exposure setting and presumed route of transmission (n=83): Sexual contact: 34 (41%, all aged over 15 years) Household contact: 19 (23%) Other: 2 (2%) Unknown: 28 (34%) 17 of the 19 household contact cases were through direct skin-to-skin contact that routinely occurs between a child and adult caregiver. In one case, fomite transmission was the suspected route of transmission as index case and child had shared a living space but had no direct skin-to-skin contact, and in one case non-household exposure when mpox positive adult held a child outside the household setting. In 2 instances the adult caregiver contracted mpox after caring for a child with mpox in household settings (skin-to-skin contact during routine childcare). No secondary transmission was identified during instances when children attended school or a childcare facility while symptomatic. Exposure setting and presumed route of transmission by age group: 0 to 4 years (n=16): Sexual contact: 0 Household contact: 13 (81%) Other: 1 (6%) Unknown: 2 (13%) 5 to 12 years (n=12): Sexual contact: 0 Household contact: 6 (50%). Other: 0 Unknown: 6 (50%) 13 to 17 years old (n=55): Sexual contact: 34 (62%, all over 15 years old) Household contact: 0 Other: 1 (2%) Unknown: 20 (36%)

Study	Country or region, time period, study type	Population	Outcomes
Silva and others, 2023 (15)	Brazil, June 12 to August 19 2022 Prospective cohort study	208 mpox cases. Median age 33 years (IQR: 28 to 38 years). 57 (39.6%) were black, 43 (29.9%) were Pardo (mixed), 43 (29.9%) were white, 1 (0.7%) indigenous 200 (96.2%) were cis-gendered men, 8 (3.8%) cis-gendered women. 156 (89.7%) MSM. 109 (60.8%) were living with HIV, 2 (1.8%) of the 109 were diagnosed at their mpox assessment. Median age 34 years (IQR: 30 to 40 years), 23 (29.5%) were black, 29 (37.2%) Pardo, 25 (32.1%) were white, 1 (1.3%) were indigenous, 108 (99.1%) were cis gender males, 1 was (90.9%) a cis gender female. Median CD4 cell count was 527.5 cells per mm^3 (IQR: 379.5, 826.7), 79 of 87 (90.8%) had undetectable HIV viral load and all were using an antiretroviral regimen. 60 of 182 (33%) with available data had at least one accompanying STI infection 31 out of 89 (31.6%) were using PrEP. 17 (8.2%) were vaccinated for smallpox	Of 156 who reported sexual contact, 35 (22.4%) reported sexual contact with a potential mpox case in the last 30 days. 11 of 146 (7.5%) cases lived in the same household as a suspected or confirmed mpox case.
Snyder and others, 2024 (16)	US, November 2022 to June 2023 Case-control study	54 mpox cases and 117 mpox negative controls. For mpox cases: 24 (44.4%) were white, 23 (42.6%) were Hispanic, 5 (9.3%) were Asian, 5 (9.3%) were black or African American, 1 (1.9%) were native Hawaiian or pacific islander, 1 (1.9%) were American Indian or Alaska native. 48 (88.9%) were cisgender men, 3 (5.6%) were cisgender women, 2 (3.7%) transgender men, 1 (1.9%) transgender women. Of those assigned male at birth (n=49), 39 (79.6%) were MSM	Overall, 17 (31.5%) of 54 cases and 7 (6.0%) of 117 case-controls reported exposure to a diagnosed or suspected index case (OR 7.3, 95% CI 2.5 to 13.4). For diagnosed index cases: 8 (14.9%) of 54 cases and 4 (3.4%) of 117 case controls reported contact with a diagnosed index case (OR 5.9, 95% CI 1.7 to 19.9), of which 2 (3.7%) of 54 cases and 2 (1.7%) of 117 case controls were nonsexual (OR 3.0, 95% CI 0.3 to 27.3).6 (11.1%) of 54 cases and 2 (1.7%) of 117 case controls were sexual contact (OR 8.9, 95% CI 2.0 to 66.3). One (1.9%) of 54 cases and one (0.9%) of 117 case controls reported sexual contact with index case with apparent symptoms at the time of their encounter (OR 3.0, 95% CI 0.1 to 111.3). 5 (9.3%) of 54 cases and 1 (0.9%) of 117 case controls reported

Study	Country or region, time period, study type	Population	Outcomes
		19 (35.2%) were living with HIV infection. CD4 counts not reported. 10 (18.5%) reported history of chlamydia, gonorrhoea, or syphilis in the last 3 weeks before mpox diagnosis. 20 (37%) had a history of JYNNEOS vaccination. 8 (14.8%) with 1 dose and 12 (22.2%) with 2 doses of the vaccine. For case controls: 67 (57.3%) were white, 48 (41.0%) were Hispanic, 10 (8.5%) were Asian, 6 (5.1%) were black, 2 (1.7%) were native Hawaiian or pacific islander, 3 (2.6%) were American Indian or Alaska native. 92 (78.6%) were cisgender men and 25 (21.4%) were cisgender women Of those assigned male at birth (n=92), 32 (34.8%) MSM 18 (15.4%) were living with HIV infection. CD4 counts not reported. 5 (4.3%) reported history of chlamydia, gonorrhoea, or syphilis in the last 3 weeks before mpox diagnosis. 27 (23.1%) had a history of JYNNEOS vaccination. 11 (9.4%) with 1 dose and 16 (13.7%) with 2 doses of the vaccine. Age of cases and case-controls not reported.	sexual contact to an index case without apparent symptoms at the time of the encounter (OR 14.9, 95% CI 2.5 to 531.8). For suspected index cases: 9 (16.7%) of 54 cases and 3 (3.2%) of 117 case controls reported contact with a diagnosed index case (OR 8.9, 95% CI 2.4 to 37.0), of which 2 (3.7%) of 54 cases and 0 of 117 case controls were nonsexual. Seven (13.0%) of 54 cases and (2.6%) of 117 case controls were sexual contact (OR 6.9, 95% CI 1.8 to 30.6). 2 (3.7%) of 54 cases and 2 (1.7%) of 117 case controls reported sexual contact with index case with apparent symptoms at the time of their encounter (OR 3.0, 95% CI 0.3 to 27.3). 5 (9.3%) of 54 cases and 1 (0.9%) of 117 case controls reported sexual contact to an index case without apparent symptoms at the time of the encounter (OR 14.9, 95% CI 2.5 to 531.8).
Van Ewijk and others, 2023 (17)	Netherlands, May 20 to August 8 2022 Prospective cohort study	1,000 mpox cases. Median age was 37 years (IQR: 31 to 45 years, range: 9 to 77 years): 0 to 17 years: 1 (0.1%) 18 to 30 years: 241 (24%) 31 to 40 years: 387 (39%) 41 to 50 years: 214 (21%) Over 50 years: 156 (16%) Unknown: 1 (NA)	227 (33%) of 678 cases had contact with an mpox case (high or medium risk contact) within 21 days of symptom onset. Cases reported a mean of 2 high risk [range 0 to 100] and 1 medium risk contact [0 to 99], where exposures were known. Reported transmission routes (n=865 cases): sexual contact: 822 (95%) direct unprotected contact: 15 (2.0%) household: 5 (0.6%) prolonged face to face contact: 20 (2.0%) other: 3 (0.4%)

Study	Country or region, time period, study type	Population	Outcomes
		Country of Origin: Netherlands: 511 (58%) Netherlands Antilles, Aruba, and Surinam: 44 (5%) Morocco: 4 (0.4%) Turkey: 2 (0.2%) Other western countries: 159 (18%) Non-western countries: 173 (19%) Unknown: 107 (NA) Sex at birth: Female: 10 (1%) Male: 987 (99%) Unknown: 3 (NA) Gender identity: Female: 7 (1%) Male: 830 (98%) Transgender man: 1 (0.1%) Transgender woman: 1 (0.1%) Other gender identity: 4 (0.5%) Unknown: 157 (NA) Sexual Orientation: MSM: 935 (95%) • 897 (94%) having sex with men • 38 (4%) having sex with men and women Sex with women: 19 (2%) Other: 2 (0.2%) Unknown: 42 (NA) 187 (21%) of 882 living with HIV. 13 (2%) were immunodeficient other than from HIV. 168 (21%) were on HIV medication. 265 (33%) of 882 were on HIV PrEP. 56 (6%) of 882 had accompanying STI.	unknown: 135 (NA)

Study	Country or region, time period, study type	Population	Outcomes
		126 (13%) of 948 were vaccinated against smallpox, before 1978. For Imvanex post-exposure prophylaxis: 869 (96%) did not receive it. 40 (4%) received it. 91 had unknown status. High risk contacts were defined as sexual contact, intensive skin to skin contact, household contact, unprotected direct contact or laboratory employees exposed to contaminated material contact with an mpox case during infectious period. Medium risk contact defined as unprotected prolonged (more than 2 hours) face to face contact within 1.5 m distance.	
Zong and others, 2023 (18)	China, May 27 to July 9 2023 Cross-sectional study	93 mpox cases reported to the National notifiable disease report system. Median age 30 (range 20 to 48; IQR 27 to 35). All were male. 89 (95.70 %) were MSM. In the last 21 days before symptom onset, 78 of 93 (83.87 %) of individuals had sex only with men, 2 out of 93 (2.15 %) had sex with both men and women, and one out of 93 (1.08%) had sex only with women. 45 (48.39%) were living with HIV. CD4 counts not reported. Ethnicity not reported.	7 clusters with 15 cases representing 15 of 93 (16.13%) mpox cases. One cluster contained 3 cases, where 2 were sexual contacts and 1 was a long-term cohabiting partner of a case. Other 6 clusters contained 2 cases, and all had sexual contact which each other. No epidemiological link was found between the remaining 78 (83.87%) cases.

Table D.4. Summary of studies measuring persistence of mpox in semen

Study	Country or region, time period, study type	Population	Outcomes
Candela and others, 2023 (23)	Italy, May to October 2022 Prospective cohort study	140 mpox cases clade not reported diagnosed at sexual health clinic. At baseline, semen samples collected from 64 men, of which 43 had DNA in seminal fluid. Of the 43 with seminal samples positive for mpox, the median age was 36 years (IQR: 34 to 42 years), 42 (98%) MSM, 12 (28%) were living with HIV and 10 (23%) had accompanying diagnosis with a STI. Ethnicity not reported	Viral DNA was detected in semen from 43 (67%) of 64 positive cases at baseline. Median Ct of viral DNA at baseline (43 participants): 34 (IQR: 31 to 36). During the 6-month follow-up, mpox viral load in semen was reassessed for 32 out of 43 participants (74%) who had previously tested positive. The other 11 participants (26%) did not submit additional samples due to painful genital lesions or penile oedema, and one individual was lost to follow-up. Viral DNA clearance at timepoints from baseline: 1 week: 19 (68%) out of 28 seminal samples tested negative for viral DNA. 2 weeks: 25 (89%) out of 28 seminal samples tested negative for viral DNA. 3 months: 26 (90%) out of 28 seminal samples tested negative for viral DNA. 6 months: 32 out of 32 (100%) seminal samples tested negative for viral DNA. Median time to viral clearance was 10.5 days (IQR: 7 to 33 days).
Mazzotta and others, 2024 (6)	Italy, May to September 2023 Retrospective cohort study	541 mpox clade IIb cases at 15 Italian health centres (median age 38 years [IQR: 33 to 44 years], 404 (74.68%) were Caucasian, 4 [0.74%] were women, 512 [94.64%] were omo-bisexual). 235 (43.44%) were living with HIV, 22 (4.07%) had CD4 count less than 350 cells per µL. 61 (11.28%) had previous smallpox vaccination	Of 28 tests of seminal fluid, after symptoms resolution, 12 (42.9%) were positive, 0 to 46 days post symptom resolution with mean Ct value of 31.66 to 40.57.
Meschi and others, 2024 (21)	Italy, May to December 2022 Retrospective cohort study	89 mpox clade IIb confirmed cases, attending the INML "L Spallanzani" in Rome, Italy. 37 mpox cases living with HIV (median CD4 count: 560.5 cell per ml^3 [IQR: 412 to 797.3]), 5 had unknown HIV status.	Sampling was taken at week 1 (7 days ± 1 day), week 2 (14 days ± 3 days), week 3 (21 days ± 3 days, week 4 (28 days ± 3 days) from enrolment. Percentage of positive semen samples detected at different weeks from symptom onset: Week 1: 64% (of 40 samples) Week 2: 74% (of 42 samples) Week 3: 38% (of 21 samples) Week 4: 32% (of 19 samples) Statistical analysis between the following time-point pairs for positive semen samples were:

		4 cases reported smallpox vaccination during childhood (median age: 62 years [IQR: 56 to 62 years]) Ethnicity, gender, and sexual orientation not reported.	week 1 and 4: p<0.001 week 2 and 3: p<0.05 week 2 and 4: p<0.005 Median Ct value for semen samples: Ct=38.3 (95% CI: 34.2 to over 40) Median time from symptom onset to viral clearance for semen samples: 14 days (95% CI: 13 to 17 days) The presence of infectious virus was assessed by recovering replication-competent virus in cell culture. A total of 11 semen samples (median Ct value: 27.9, IQR 25.2 to 29.5; median days since symptom onset: 10, IQR 7.5 to 11.5 days) were collected from 10 patients on different days. Replication-competent virus was successfully isolated from 2 of these semen samples, each from different patients. The Ct values for the positive isolates were 22.7 and 29.3, with the days since symptom onset being 4 and 12, respectively.
Piralla and others, 2024 (22)	Italy, May 24 to September 1 2022. Retrospective cohort study	353 mpox cases clade IIb. Median age 37 years, range 15 to 67 years [IQR: 32 to 43 years] 99.2% male, 10.5% living with HIV, 84.7% unknown HIV status). Of 261 cases reporting vaccination history for smallpox, 231 (65.4%) individuals were unvaccinated. Ethnicity and sexual orientation not reported. Participants were sampled up to 56 days post presentation.	At diagnosis, 37 of 77 semen samples taken (48.1%) were positive for mpox. Median clearance time of mpox DNA was 7 days in semen samples (of 24 samples). Replication-competent virus was isolated in 100% (3 out of 3) seminal specimens supporting the evidence of infectiousness of mpox virus in semen. A total of 329 (93.2%) exposure histories were available, of which: 244 (74.1%) of them were autochthonous (indigenous) cases 85 (25.8%) were cases where transmission likely occurred abroad (Spain, France, Germany, Great Britain) 247 (69.8%) were defined as local transmission cases, 86 (24.3%) were not, and 21 (5.9%) were unknown.
Raccagni 2024 (24)	Italy, May to November 2023 Retrospective cohort study	95 laboratory confirmed mpox cases clade not reported. Median age: 39.4 years (IQR: 35.4 to 44.7 years). All were MSM. HIV status: • People living with HIV: 50 out of 95 (52.6%) • 44 out of 50 (89.8%) had an HIV-RNA level less than 50 copies per mL	Median number of days with detectable mpox virus in semen was 8 days (IQR: 7 to 15 days)

		• Median CD4 cell count at the time of mpox infection: 690 cells per µL (IQR: 559 to 1,005 cells per µL) People using PrEP: 33 out of 95 (34.7%) Vaccination status: • 16 out of 95 (16.84%) reported having received smallpox vaccination during their youth Samples were taken every 7 days and tested with PCR until the end of infection	
Suner and others, 2023 (25)	Spain, June 28 to September 2022 Prospective cohort study	77 mpox cases clade not reported. 75 (97%) were men, one (1%) transgender woman and one (1%) female, median age was 35.0 years (IQR: 29·0 to 46·0 years). 36 (47%) were Spanish, 31 (40%) were from south and Latin America, 9 (12%) were other European countries, one (1%) were west African. 3 (4%) were bisexual men, 2 (3%) were heterosexual men, 2 (3%) were heterosexual women and 70 (91%) were MSM. 39 (51%) of 77 participants were living with HIV. No participants had a diagnosis of accompanying STI	Time to viral clearance in semen for 50% of patients (95% CI): 13 days (9 to 18 days) Time to viral clearance in semen for 90% of patients (95% CI): 39 days (27 to 56 days) Time to viral clearance in semen for 95% of patients (95% CI): 53 days (34 to 84 days) The median time of viral clearance was 13 days (95% CI 9 to 18 days). Time to viral clearance by HIV status [data estimated from figure]: Living with HIV: 13.0 days Living without HIV: 18.9 days This difference is statistically significant (p=0.0043). Median viral load at baseline for semen was 3.5 log_{10} copies per mL (IQR 2.9 to 4.7). 1 sample shed viral DNA for more than 57 days (4 log_{10} copies per mL). 3 (1%) of 219 semen samples had a viral load of 6.5 log_{10} copies per mL or higher. It took 2 days (95% CI 0 to 11) for viral load to fall below 6.5log_{10} copies per mL in 90% of patients, and 8 days (95% CI: 0 to 19 days) for 95% of patients. 2 semen samples tested for viral culture, 1 was positive. Proportion of samples positive at different timepoints from symptom onset for semen [Data extracted from figure]:

			1 to 5 days: 63% (95% CI: 41 to 83) 6 to 10 days: 71% (95% CI: 50 to 86) 11 to 15 days: 44% (95% CI: 14 to 79) 16 to 20 days: 23% (95% CI: 11 to 44) 21 to 25 days: 32% (95% CI: 18 to 50) Over 25 days: 4% (95% CI: 1 to 10)

Table D.5. Summary of studies measuring environmental samples

Study	Country, time period, study type	Population	Outcomes
Gould and others, 2022 (27)	UK, May 24 to June 17 2022 Cross-sectional study.	Isolation rooms of 7 hospitalised adults with confirmed mpox (clade not reported) and active skin lesions were sampled. Samples were taken from surfaces in patients' room, bathroom, anteroom, PPE, and air. The air samples were taken before and after bed changes, and before and during doffing of PPE. Rooms were positive-pressure ventilated lobby, single-occupancy respiratory isolation rooms. Rooms had a minimum of 10 air changes per hour and were cleaned every 12 hours. Ethnicity, age, sex, HIV status and sexual orientation of room occupants were not reported.	Mpox DNA was found in 56 (93%) of surface swabs obtained within patients' bedrooms and bathrooms with Ct values ranging from 24.7 to 37.4. Ct values of surfaces in patient rooms likely touched by mpox case (n=5 rooms): Floor: positive for mpox in 4 out of 5 rooms (80%). Ct value range: 26.9 to 34.9 Call button: positive for mpox in 4 out of 5 rooms (80%). Ct value range: 26.1 to 32.4 Light switch: positive for mpox in 5 out of 5 rooms (100%). Ct value range: 24.7 to 36.3 TV remote control: positive for mpox in 5 out of 5 rooms (100%). Ct value range: 25.0 to 37.4 Observation machine: positive for mpox in one out of 5 rooms (20%). Ct value: 26.4 Tap handle: positive for mpox in 5 out of 5 rooms (100%). Ct value range: 27.1 to 36.7 Deposition area (window ledge): positive for mpox in 4 out of 5 rooms (80%). Ct value range: 28.8 to 35.6 Chair (armrest): positive for mpox in 5 out of 5 rooms (100%). Ct value range: 24.9 to 33.8 Door handle (patient room to bathroom): positive for mpox in 4 out of 5 rooms (80%). Ct value range: 26.7 to 33.3 Toilet flush handle: positive for mpox in 5 out of 5 rooms (100%). Ct value range: 26.4 to 34.8 Shower handle: positive for mpox in 5 out of 5 rooms (100%). Ct value range: 28.8 to 34.0 Tap handle (bathroom): positive for mpox in 4 out of 5 rooms (80%). Ct value range 25.9 to 32.8 Ct values of surfaces unlikely touched by mpox case (n=5 rooms): Bathroom vent or grille (room to bathroom): positive for mpox in 5 out of 5 rooms (100%). Ct value range: 25.9 to 33.6 Anteroom floor, toxic side (close to patient): positive for mpox in 5 out of 5 rooms (100%). Ct value range 26.3 to 33.2 Anteroom floor, non-toxic side (close to the exit): positive for mpox in 3 out of 5 rooms (60%). Ct value range 33.6 to 36.8 Floor in ward corridor: positive for mpox in 2 out of 5 rooms (40%). Ct value range 36.7 to 37.5 Samples from PPE (n=12): 4 (33.3%) samples positive for mpox.

Study	Country, time period, study type	Population	Outcomes
			Samples from gloves (n=3): 2 (66.7%) samples positive for mpox (Ct values 30.8 and 27.1 were taken where swabbing included the palmar surface and fingertips, but not detected on sample collected by swabbing palmar surface only) Gowns (n=3): 2 (66.7%) samples positive for mpox (Ct values: 35.6 and 34.3) Anteroom floor, after removing PPE (n=3): 3 samples positive for mpox (Ct values: 26.1, 26.9 and 30.9) Visor (n=3): all samples negative Air sampling: Near patient bed (within 1m), before bedding change: all values were negative. Near patient bed (within 1m), during bedding change: 2 out of 5 samples positive. Ct values 32.7 and 36.2 In patient room (more than 1.5m away from bed), before bedding change: 2 out of 5 samples positive. Ct values 36.2 and 36.5 In patient room (more than 1.5m away from bed), during bedding change: 1 out 5 samples positive. Ct value 35.8 In corridor, before putting on PPE: 1 out of 3 samples positive for mpox (Ct value of 38.2) In corridor, while putting on PPE: negative for all rooms Anteroom, before putting on PPE: negative for all rooms Anteroom, while putting on PPE: negative for all rooms
Hernaez and others, 2023 (28)	Spain, May 23 to September 2022 Cross-sectional study	44 symptomatic mpox cases (clade not reported). Median age (35 years [IQR: 11.3 years]). All cis-gendered men, 41 (94%) MSM. 23 (52%) cases were living with HIV and on treatment, all had undetectable viral load. 12 (27%) of patients had an accompanying STI other than HIV. 11 (25%) received the smallpox vaccine. 6 (14%) did not know vaccination status. Ethnicity was not reported. Collected exhaled breath, saliva and respiratory tract secretion and air samples	Exhaled breath samples through mask (n=45): 32 (71%) of mask filters were positive for mpox by qPCR (Ct value less than 35). Lowest Ct value was 26. Viral load detected in positive mask filters ranged from 70 to $6x10^4$ mpox virus genomes per mask. The number of positive mask filter samples where infectious virus was recovered was 2. Both samples with infectious viruses were in the 85th percentile of viral load. Air samples (n=42): 27 (64%) air filters tested positive for mpox by qPCR. This is despite cases wearing a FFP2 face mask during testing. Lowest Ct value 29. The viral loads calculated ranged from 40 to about $9×10^3$ monkeypox virus genomes per m^3. Viral load detected in positive mask filters ranged from 70 to 6x10^4 mpox virus genomes per mask. The number of positive mask filter samples where infectious virus was recovered was 2. Both samples with infectious viruses were in the 85th percentile of viral load. Saliva samples (n=41)

Study	Country, time period, study type	Population	Outcomes
		taken from 2 to 3 m from and 1.5m above the patients.	35 (85%) of samples tested positive for mpox by qPCR. Lowest positive Ct value was 18. Most Ct values ranged from 20 to 26 (full range: 18 to 38 [range data extracted from figure]). Ct values were higher than those detected in air samples. Cells inoculated with saliva showed mpox virus induced cytopathic effect at the first attempt in 22(67%) of the 33 positive samples. The cytopathic effect spread rapidly through the cell culture in the first 5 days, indicating presence of infectious virus in the saliva. Recovering infectious viruses was more likely in samples with Ct lower than 26 or with a viral load higher than 10^4 monkeypox virus genomes per mL of saliva
Yang and others, 2024 (26)	China, June 11 to November 13 2023 Prospective cohort study.	77 hospitalised mpox cases (clade IIb). Median age 30 years (IQR: 21 to 51 years). All were men. 72 (93.5%) MSM, 5 (6.5%) bisexual. 42 (54.5%) were people living with HIV with a median CD4 count 450 (IQR: 237 to 566), and the rest were immunocompetent. 5 (6.5%) cases received smallpox vaccination during childhood. Ethnicity was not reported.	1,633 environmental swabs were taken from 49 cases hospital rooms. Overall 860 of 1,633 (52.66%) of environmental fomite swabs were positive for mpox DNA. Percentage of swabs from patient rooms positive for mpox: deposition area (air conditioning air outlet): 69.89% (65 out of 93) pillow: 68.0% (85 out of 125) floor: 62.9% (56 out of 89) bedside cupboard: 61.6% (77 out of 125) bed handrail: 59.7% (74 out of 124) clothes: 56.8% (71 out of 125) chair (arm rest): 54.2% (58 out of 107) mobile phone: 52.8% (66 out of 125) shower handle: 47.4% (45 out of 95) toilet flush handle: 45.7% (43 out of 94) door handle (patient room to bathroom): 45.2% (42 out of 93) television remote control: 44.7% (42 out of 94) call button: 43.2% (54 out of 125) delivery window: 37.9% (36 out of 95) light switch: 37.1% (46 out of 124) The mean viral loads of these samples were 5.37 log_{10} copies per mL (Ct value: 32.83). Differences were found among different swabs (ANOVA, p = 0.008), with the mean viral loads in the deposition area (5.82 log_{10} copies per mL) being the highest. Viral loads of surfaces in patient rooms, and distribution of mpox viral loads on different surfaces over time [distribution over time estimated from figure]: Floor: Overall: Median viral load 5.54 log_{10} copies per mL (IQR: 5.06 to 6.26)

Study	Country, time period, study type	Population	Outcomes
			1 to 7 days post-symptom onset: Median viral load 5.21 log_{10} copies per mL (IQR: 5.08 to 5.92). 25.2% samples negative for mpox, 65.3% viral load lower than 6.59 log_{10} copies per mL, 9.5% viral load higher than 6.59 log_{10} copies per mL. 8 to 14 days post-symptom onset: Median viral load 5.56 log_{10} copies per mL (IQR: 4.96 to 6.27). 53.1% samples negative for mpox, 40.1% viral load lower than 6.59 log_{10} copies per mL, 6.8% viral load higher than 6.59 log_{10} copies per mL. 15 to 21 days post-symptom onset: Median viral load 5.86 log_{10} copies per mL (IQR: 5.26 to 6.65). 22.5% samples negative for mpox, 50.3% viral load lower than 6.59 log_{10} copies per mL, 27.2% viral load higher than 6.59 log_{10} copies per mL. Call button: Overall: Median viral load 5.39 log_{10} copies per mL (IQR: 4.87 to 6.16) 1 to 7 days post-symptom onset: Median viral load 5.45 log_{10} copies per mL (IQR: 4.89 to 5.85). 60.5% samples negative for mpox, 36.1% viral load lower than 6.59 log_{10} copies per mL, 3.4% viral load higher than 6.59 log_{10} copies per mL. 8 to 14 days post-symptom onset: Median viral load 5.30 log_{10} copies per mL (IQR: 4.81 to 6.07). 61.2% samples negative for mpox, 34.7% viral load lower than 6.59 log_{10} copies per mL, 4.1% viral load higher than 6.59 log10 copies per mL. 15 to 21 days post-symptom onset: Median viral load 5.75 log_{10} copies per mL (IQR: 5.17 to 6.39). 53.7% samples negative for mpox, 42.2% viral load lower than 6.59 log_{10} copies per mL, 4.1% viral load higher than 6.59 log_{10} copies per mL. Light switch: Overall: Median viral load 4.85 log_{10} copies per mL (IQR: 4.57 to 5.43) 1 to 7 days post-symptom onset: Median viral load 5.20 log_{10} copies per mL (IQR: 4.60 to 5.40). 69.4% samples negative for mpox, 24.5% viral load lower than 6.59 log_{10} copies per mL, 6.1% viral load higher than 6.59 log_{10} copies per mL. 8 to 14 days post-symptom onset: Median viral load 4.84 log_{10} copies per mL (IQR: 4.56 to 5.29). 63.3% samples negative for mpox, 36.7% viral load lower than 6.59 log_{10} copies per mL, 0 viral load higher than 6.59 log_{10} copies per mL. 15 to 21 days post-symptom onset: Median viral load 5.14 log_{10} copies per mL (IQR: 4.57 to 5.50). 62.6% samples negative for mpox, 33.3% viral load lower than 6.59 log_{10} copies per mL, 4.1% viral load higher than 6.59 log_{10} copies per mL. Television remote Overall: Median viral load 5.26 log_{10} copies per mL (IQR: 4.85 to 5.84) 1 to 7 days post-symptom onset: Median viral load 4.90 log_{10} copies per mL (IQR: 4.53 to 5.20). 71.4% samples negative for mpox, 28.6% viral load lower than 6.59 log_{10} copies per mL, 0 viral load higher than 6.59 log_{10} copies per mL.

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			8 to 14 days post-symptom onset: Median viral load 5.28 log_{10} copies per mL (IQR: 4.83 to 5.90). 55.1% samples negative for mpox, 42.9% viral load lower than 6.59 log_{10} copies per mL, 2.0% viral load higher than 6.59 log_{10} copies per mL. 15 to 21 days post-symptom onset: Median viral load 5.38 log_{10} copies per mL (IQR: 5.07 to 5.95). 39.5% samples negative for mpox, 55.1% viral load lower than 6.59 log_{10} copies per mL, 5.4% viral load higher than 6.59 log_{10} copies per mL. Bed rail Overall: Median viral load 4.93 log_{10} copies per mL (IQR: 4.69 to 5.64) 1 to 7 days post-symptom onset: Median viral load 4.94 log_{10} copies per mL (IQR: 4.73 to 5.71). 48.3% samples negative for mpox, 48.3% viral load lower than 6.59 log_{10} copies per mL, 3.4% viral load higher than 6.59 log_{10} copies per mL. 8 to 14 days post-symptom onset: Median viral load 5.00 log_{10} copies per mL (IQR: 4.71 to 5.59). 40.1% samples negative for mpox, 52.4% viral load lower than 6.59 log_{10} copies per mL, 7.5% viral load higher than 6.59 log_{10} copies per mL. 15 to 21 days post-symptom onset: Median viral load 4.89 log_{10} copies per mL (IQR: 4.56 to 5.61). 56.5% samples negative for mpox, 38.7% viral load lower than 6.59 log_{10} copies per mL, 4.8% viral load higher than 6.59 log_{10} copies per mL. Bedside cupboard Overall: Median viral load 5.47 log_{10} copies per mL (IQR: 4.73 to 6.35) 1 to 7 days post-symptom onset: Median viral load 5.57 log_{10} copies per mL (IQR: 4.71 to 6.14). 60.0% samples negative for mpox, 33.9% viral load lower than 6.59 log_{10} copies per mL, 6.1% viral load higher than 6.59 log_{10} copies per mL. 8 to 14-days post-symptom onset: Median viral load 5.72 log_{10} copies per mL (IQR: 4.84 to 6.49). 35.4% samples negative for mpox, 51.7% viral load lower than 6.59 log_{10} copies per mL, 12.9% viral load higher than 6.59 log_{10} copies per mL. 15 to 21 days post-symptom onset: Median viral load 5.02 log_{10} copies per mL (IQR: 4.63 to 6.00). 29.3% samples negative for mpox, 58.5% viral load lower than 6.59 log_{10} copies per mL, 12.2% viral load higher than 6.59 log_{10} copies per mL. Chair (arm rest): Overall: Median viral load 5.02 log_{10} copies per mL (IQR: 4.77 to 5.64) 1 to 7 days post-symptom onset: Median viral load 4.89 log_{10} copies per mL (IQR: 4.46 to 5.10). 59.9% samples negative for mpox, 36.7% viral load lower than 6.59 log_{10} copies per mL, 3.4% viral load higher than 6.59 log_{10} copies per mL. 8 to 14 days post-symptom onset: Median viral load 5.31 log_{10} copies per mL (IQR: 4.64 to 5.67). 48.3% samples negative for mpox, 50.3% viral load lower than 6.59 log_{10} copies per mL, 1.4% viral load higher than 6.59 log_{10} copies per mL.

Study	Country, time period, study type	Population	Outcomes
			15 to 21 days post-symptom onset: Median viral load 5.30 log_{10} copies per mL (IQR: 4.93 to 5.90). 36.7% samples negative for mpox, 48.3% viral load lower than 6.59 log_{10} copies per mL, 15.0% viral load higher than 6.59 log_{10} copies per mL. Door handle (patient room to bathroom) Overall: Median viral load 5.34 log_{10} copies per mL (IQR: 4.90 to 6.03) 1 to 7 days post-symptom onset: Median viral load 5.03 log_{10} copies per mL (IQR: 4.94 to 5.22). 63.3% samples negative for mpox, 36.7% viral load lower than 6.59 log_{10} copies per mL, 0 viral load higher than 6.59 log_{10} copies per mL. 8 to 14 days post-symptom onset: Median viral load 5.27 log_{10} copies per mL (IQR: 4.84 to 6.17). 59.2% samples negative for mpox, 32.0% viral load lower than 6.59 log_{10} copies per mL, 8.8% viral load higher than 6.59 log_{10} copies per mL. 15 to 21 days post-symptom onset: Median viral load 6.01 log_{10} copies per mL (IQR: 5.13 to 6.04). 53.1% samples negative for mpox, 35.3% viral load lower than 6.59 log_{10} copies per mL, 11.6% viral load higher than 6.59 log_{10} copies per mL. Deposition area (air conditioning air outlet) Overall: Median viral load 5.53 log_{10} copies per mL (IQR: 5.05 to 6.52) 1 to 7 days post-symptom onset: Median viral load 5.45 log_{10} copies per mL (IQR: 5.17 to 6.25). 41.5% samples negative for mpox, 49.0% viral load lower than 6.59 log_{10} copies per mL, 9.5% viral load higher than 6.59 log_{10} copies per mL. 8 to 14 days post-symptom onset: Median viral load 5.47 log_{10} copies per mL (IQR: 5.01 to 6.25). 29.9% samples negative for mpox, 52.4% viral load lower than 6.59 log_{10} copies per mL, 17.7% viral load higher than 6.59 log_{10} copies per mL. 15 to 21 days post-symptom onset: Median viral load 5.95 log_{10} copies per mL (IQR: 5.17 to 6.91). 29.3% samples negative for mpox, 49.6% viral load lower than 6.59 log_{10} copies per mL, 21.1% viral load higher than 6.59 log_{10} copies per mL. Mobile phone Overall: Median viral load 5.22 log_{10} copies per mL (IQR: 4.84 to 5.83) 1 to 7 days post-symptom onset: Median viral load 5.04 log_{10} copies per mL (IQR: 4.84 to 5.33). 58.5% samples negative for mpox, 38.8% viral load lower than 6.59 log_{10} copies per mL, 2.7% viral load higher than 6.59 log_{10} copies per mL. 8 to 14 days post-symptom onset: Median viral load 5.23 log_{10} copies per mL (IQR: 4.78 to 5.89). 49.0% samples negative for mpox, 43.5% viral load lower than 6.59 log_{10} copies per mL, 7.5% viral load higher than 6.59 log_{10} copies per mL. 15 to 21 days post-symptom onset: Median viral load 5.31 log_{10} copies per mL (IQR: 4.92 to 5.84). 53.3% samples negative for mpox, 41.5% viral load lower than 6.59 log_{10} copies per mL, 8.2% viral load higher than 6.59 log_{10} copies per mL.

Study	Country, time period, study type	Population	Outcomes
			Clothes Overall: Median viral load 5.12 log_{10} copies per mL (IQR: 4.68 to 5.79) 1 to 7 days post-symptom onset: Median viral load 5.06 log_{10} copies per mL (IQR: 4.58 to 5.29). 64.6% samples negative for mpox, 32.0% viral load lower than 6.59 log_{10} copies per mL, 3.4% viral load higher than 6.59 log_{10} copies per mL. 8 to 14 days post-symptom onset: Median viral load 5.16 log_{10} copies per mL (IQR: 4.76 to 5.86). 38.8% samples negative for mpox, 55.8% viral load lower than 6.59 log_{10} copies per mL, 5.4% viral load higher than 6.59 log_{10} copies per mL. 15 to 21 days post-symptom onset: Median viral load 5.15 log_{10} copies per mL (IQR: 4.89 to 5.79). 41.5% samples negative for mpox, 50.3% viral load lower than 6.59 log_{10} copies per mL, 8.2% viral load higher than 6.59 log_{10} copies per mL. Pillow Overall: Median viral load 5.32 log_{10} copies per mL (IQR: 4.85 to 6.07) 1 to 7 days post-symptom onset: Median viral load 5.08 log_{10} copies per mL (IQR: 4.50 to 6.00). 46.9% samples negative for mpox, 42.2% viral load lower than 6.59 log_{10} copies per mL, 10.9% viral load higher than 6.59 log_{10} copies per mL. 8 to 14 days post-symptom onset: Median viral load 5.36 log_{10} copies per mL (IQR: 4.94 to 6.15). 28.6% samples negative for mpox, 59.2% viral load lower than 6.59 log_{10} copies per mL, 12.2% viral load higher than 6.59 log_{10} copies per mL. 15 to 21 days post-symptom onset: Median viral load 4.98 log_{10} copies per mL (IQR: 4.66 to 5.85). 36.1% samples negative for mpox, 55.1% viral load lower than 6.59 log_{10} copies per mL, 8.8% viral load higher than 6.59 log_{10} copies per mL. Toilet flush handle Overall: Median viral load 5.12 log_{10} copies per mL (IQR: 4.74 to 6.01) 1 to 7 days post-symptom onset: Median viral load 5.54 log_{10} copies per mL (IQR: 4.85 to 5.88). 66.0% samples negative for mpox, 28.6% viral load lower than 6.59 log_{10} copies per mL, 5.4% viral load higher than 6.59 log_{10} copies per mL. 8 to 14 days post-symptom onset: Median viral load 5.13 log_{10} copies per mL (IQR: 4.71 to 6.05). 53.7% samples negative for mpox, 36.8% viral load lower than 6.59 log_{10} copies per mL, 9.5% viral load higher than 6.59 log_{10} copies per mL. 15 to 21 days post-symptom onset: Median viral load 4.92 log_{10} copies per mL (IQR: 4.90 to 5.80). 43.5% samples negative for mpox, 51.1% viral load lower than 6.59 log_{10} copies per mL, 5.4% viral load higher than 6.59 log_{10} copies per mL. Shower handle Overall: Median viral load 5.24 log_{10} copies per mL (IQR: 4.87 to 5.64)

Study	Country, time period, study type	Population	Outcomes
			1 to 7 days post-symptom onset: Median viral load 5.45 log_{10} copies per mL (IQR: 5.23 to 5.61). 52.4% samples negative for mpox, 43.5% viral load lower than 6.59 log_{10} copies per mL, 4.1% viral load higher than 6.59 log_{10} copies per mL. 8 to 14 days post-symptom onset: Median viral load 5.18 log_{10} copies per mL (IQR: 4.64 to 5.49). 54.4% samples negative for mpox, 45.6% viral load lower than 6.59 log_{10} copies per mL, 0 viral load higher than 6.59 log_{10} copies per mL. 15 to 21 days post-symptom onset: Median viral load 5.30 log_{10} copies per mL (IQR: 5.00 to 5.67). 53.1% samples negative for mpox, 46.9% viral load lower than 6.59 log_{10} copies per mL, 0 viral load higher than 6.59 log_{10} copies per mL. Delivery window Overall: Median viral load 5.17 log_{10} copies per mL (IQR: 4.74 to 5.63) 1 to 7 days post-symptom onset: Median viral load 4.69 log_{10} copies per mL (IQR: 4.53 to 5.04). 81.6% samples negative for mpox, 18.4% viral load lower than 6.59 log_{10} copies per mL, 0 viral load higher than 6.59 log_{10} copies per mL. 8 to 14 days post-symptom onset: Median viral load 5.17 log_{10} copies per mL (IQR: 4.74 to 5.69). 56.5% samples negative for mpox, 42.1% viral load lower than 6.59 log_{10} copies per mL, 1.4% viral load higher than 6.59 log_{10} copies per mL. 15 to 21 days post-symptom onset: Median viral load 5.34 log_{10} copies per mL (IQR: 4.92 to 5.83). 57.8% samples negative for mpox, 32.0% viral load lower than 6.59 log_{10} copies per mL, 10.2% viral load higher than 6.59 log_{10} copies per mL. For all sites, no statistically significant difference for median viral load between 1 to 7 days post-symptom onset, 8 to 14 days post-symptom onset or 15 to 21 days post-symptom onset (all $p > 0.05$). The proportion of swabs with viral loads higher than 6.59 log_{10} copies per mL was highest for the deposition area (17.2%), followed by the bedside cupboard (11.2%), floor (11.83%), and then the pillow (10.4%).

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