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SHORT COMMUNICATION

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Monkeypox viral detection in semen specimens of confirmed cases: A systematic review and meta-analysis

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Abstract

The current literature shows increasing concerns about potential seminal transmission of monkeypox virus (MPXV). Accordingly, we aimed to understand better the potential presence of MPXV in the seminal fluids and others specimens obtained from MPX cases. On June 26, 2022, a systematic search of the literature was conducted to find articles that examine the presence of MPXV in the seminal fluid of confirmed cases. The search was updated once on August 12 and another on October 12, 2022, to include newly published articles. The prevalence of MPXV DNA presence in the seminal fluid and other specimens was pooled in a meta-analysis (from studies with sample size > 5 to reduce overestimation) and results were presented as effect sizes (ES) and their corresponding 95% confidence intervals (CI). Nine articles were included. Only five studies were eligible for a meta-analysis, and the pooled prevalence of MPXV DNA in semen specimens was 72.4% (95% CI: 55.7%−84.5%) among 115 patients. The positive rate of MPXV viral polymerase chain reaction (PCR) was higher among skin samples (89%; 95% CI: 78.2%−94.8%; $N = 62$; studies = 2), followed by anogenital/rectal samples (74.3%; 95% CI: 60.4% −84.5%; $N = 54$; studies = 2). On the other hand, the positivity rate was lower in nasopharyngeal (62.4%; 95% CI: 20.4%−91.5%; $N = 587$; studies = 3), urine (21.1%; 95% CI: 4.3%−61.1%; $N = 617$; studies = 4), and blood/plasma (14.3%; 95% CI: 11.3%−18.1%; $N = 609$; studies = 3) samples. Besides, MPXV can be detected in semen early from Day 1 and up to 19 days after symptoms onset. Finally, two articles investigated the infectivity of MPXV particles detected in seminal specimens by testing their replication competence. Culturing MPXV was successful in two out of four patients included in these studies. MPXV is highly prevalent in seminal specimens of MPX cases, further corroborating the role of sexual transmission of the disease. However, further evidence is still needed to shed more light on the replication competence of these particles.

KEYWORDS

monkeypox, semen, seminal, sexual, viral detection

Abdullah Reda and Abdelaziz Abdelaal are contributed equally to the study.

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1 | INTRODUCTION

Due to the current, rapid, and widespread monkeypox virus (MPXV), the World Health Organization declared this multi-country outbreak a public health emergency of international concerns. On October 11, 2022, the number of confirmed MPX cases reached 71 408 cases in 107 countries, being the highest in the United States (26 778 cases), Brazil (8270), Spain (7219 cases), France (4043), the United Kingdom (3654), and Germany (3651 cases), respectively.[1]

Evidence from previous MPXV outbreaks shows that the disease is characterized by the widespread of characteristic rash with multiple lesions affecting different body parts, including the legs, arms, and face, and less commonly, in the soles, palms, and genitalia.[2–4] On the other hand, evidence from the current outbreak shows that the rash shows an atypical pattern to the previously reported one, spreading mainly on the genital and perianal regions.[5,6] Such events have been concerning since different reports indicated that most MPXV cases are individuals that identify themselves as men who have sex with men (MSM).[7–9] Therefore, the widespread of rashes and MPXV lesions in the genital and perianal regions might suggest viral transmission in this population. The transmission of MPXV has been recorded in different ways, including close contact with MPXV cases (mainly when contacting an MPXV active lesion), contacting animals directly or infected materials, and prolonged contact with infected individuals by droplet transmission.[9–12]

Moreover, there have been concerns about the potential viral transmission among seminal fluids since most cases were reported among MSM. However, no cumulative evidence was found in the literature to indicate this hypothesis, and data is scattered among single reports with insufficient highlights regarding positive MPXV DNA in seminal specimens.[6,8,13–15] Accordingly, we aimed to conduct the current systematic review to understand better the potential presence of MPXV in the seminal fluids of infected patients.

2 | METHODS

2.1 | Study design and search strategy

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines,[16] where prior registration of a review protocol is not mandatory. On June 26, 2022, eight databases were searched including PubMed, Scopus, Web of Science, EMBASE, ScienceDirect, ProQuest, EBSCOHost-Academic Search Complete, and Google Scholar. In this regard, the following keywords were used to retrieve relevant articles: monkeypox AND (semen OR seminal). We also used Medical Subject Headings terms whenever possible. The search keywords were adjusted as per the database's guidelines. A full description of the used search strategy in each database is provided in (Supporting Information: Table 1). Noteworthy, the database search was updated once on August 12 and another on October 12, 2022. Additionally, we adopted a manual search strategy to retrieve any relevant article that was missed during the electronic database search. This strategy was conducted by: (1) screening the references of included studies, (2) searching "similar articles" of finally included studies on PubMed, and (3) conducting a random search on Google engine with the keywords "monkeypox" + "semen." No restrictions regarding year, country, or the language of publication were applied.

2.2 | Eligibility criteria

This systematic review was conducted according to the population, intervention, comparison, outcome framework, where the population included MPXV cases, no interventions, no comparisons, and the primary outcome included estimating the positivity rate of MPXV in collected semen specimens. Secondary outcomes included (1) estimating the time interval between symptom onset and MPXV DNA positivity in seminal specimens and (2) estimating the positivity rate of MPXV in other collected specimens (urine, fecal, oropharynx, genitalia, and blood/plasma).

Since we aim to investigate the presence and prevalence of MPXV in the seminal specimens of MPXV cases, we included studies that (1) were original regardless of their design (i.e., case reports and series, cohort, cross-sectional studies), (2) included human individuals, (3) examined the presence of MPXV in the seminal fluids of infected MPXV cases. On the contrary, we excluded studies that (1) were not original (reviews, editorials, and letters to editors with no original data, commentaries, thesis, abstract-only articles, and posters), (2) animal, in vivo, and in vitro studies, (3) did not collect seminal specimens from recruited cases, (4) did not include MPXV cases, (5) included overlapping/non-extractable datasets of MPXV, and (5) duplicated records.

2.3 | Screening and study selection

After completing the search strategy, a senior member collected all the search results into a single Endnote library, which was used to remove all duplicates among the various databases considered in the search strategy. An excel sheet was then drafted with article reference, title, abstract, DOI, URL, journal, and link to full-text to start the screening process, which was done in two steps: title/abstract and full-text screening. These steps were done in a blind approach by at least two reviewers who discussed their differences to reach a final decision under the supervision of the senior authors. Finally, all included studies were grouped and prepared for data extraction.

2.4 | Extraction and quality assessment

Data extraction was conducted in a similar approach to that of screening. A senior author drafted a pilot sheet on Excel that included three main tabs, a baseline characteristics tab, another

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one for intended outcomes, and the third one for quality assessment. Extracted baseline characteristics included reference, country, study design, sample size, age, gender, symptoms, travel history, previous smallpox vaccination, sexual transmission (through MSM or not), and diagnostic method. The outcome tab included events of sexually transmitted infections, lesion site, and diagnostic sample (including semen, urine, blood/plasma, skin, genital/anal, fecal, and oral/nasopharyngeal). Events of positive MPXV DNA, time of onset of symptoms, and viral loads were extracted for each of these samples.

The quality of included case series was assessed using the National Institute of Health (NIH) quality assessment tool. The tool assesses the quality of each study at the level of seven domains/questions. Each domain, as well as the overall quality, is given a rating of good, fair, or poor. Two reviewers assessed the quality of included studies, and any discrepancies among them were solved by consulting one of the senior authors.

2.5 | Data synthesis

The quantitative synthesis was conducted using STATA Software (Version 17). The overall prevalence of positivity rate of MPXV viral PCR in different specimens was estimated using the metaprop command. The random-effects and fixed-effects models were used according to the presence or absence of heterogeneity, respectively. Heterogeneity was measured using the I^2 statistic, where a value of >50% or a p-value of <0.05 indicates significant heterogeneity. The exact cimethod was used to pool the ES along with its 95% confidence interval (CI). The assessment of publication bias was not feasible due to the lack of a sufficient number of studies (10 studies).

3 | RESULTS

3.1 | Search results

The results of the initial and updated database searches are illustrated in Figure 1. The initial database search yielded 573 articles, out of which 70 duplicates were identified and excluded through EndNote Software. The title and abstracts of 503 articles were screened, resulting in 13 articles eligible for full-text screening. Three studies were included with the initial database search,[8,14,17] five were added with the updated search on August 12, 2022,[6,13,15,18,19] one was added on October 12, 2022,[20] and none was added through manual search. We furtherly found that Angelo et al.[21] and Hornuss et al.[22] reported positive seminal samples in their cohort. However, the exact rate of these samples was not provided. So, we excluded them from our study. Finally, nine studies were included in the qualitative synthesis and five studies were included in the quantitative synthesis.

3.2 | Baseline characteristics

The baseline characteristics of included studies are presented in Table 1. Six studies were case series[6,8,14,15,18,20] and three were case reports.[13,17,19] The number of included patients in each study ranged from one[13,17,19] to as high as 528 MPX-confirmed cases.[6] Most cases were adults with age > 18 years. In terms of presenting symptoms and/or complaints, the majority of patients presented with rash, fever, lymphadenopathy, lethargy, myalgia, or headache. Six studies reported the history of travel of MPX-confirmed cases, where 158 cases (out of 549, 28.77%) reported traveling abdroad in the past few months before contracting the infection. A minority of patients reported being previously vaccinated with smallpox vaccines (61 out of 597 MPX-confirmed cases). MSM was evident in almost all of the reported cases, where six studies reported that all of these cases were MSM.[8,13,15,17–19] Meanwhile, in the largest case series of Thornhill et al. 99.80% (509 out of 528) of MPX-confirmed cases were MSM.[6] As for the diagnostic method used to confirm the diagnosis of MPX, reverse-trascriptase-PCR was the diagnostic method reported in all of the included studies.

The history of previous or concurrent sexually-transmitted infections (STIs) among MPX-confirmed cases in included studies is highlighted in Table 2. These STIs included human immunodeficiency virus (overall, 265/635 cases),[6,8,13–15,18–20] Herpes simplex virus (4/378 cases),[6,19] Chlamydia trachomatis (21/389),[6,15] Neisseria gonorrhea (33/389),[6,15] and syphilis (39/394).[6,15]

3.3 | Quality assessment

The quality of included studies across different domains is presented in Table 3. Overall, six case series were assessed,[6,8,14,15,18,20] all of which had good quality.

3.4 | The positivity rate of MPXV DNA in seminal specimens

Out of nine studies, five were eligible for a meta-analysis (examined at least 5 semen specimens of MPXV cases).[6,13,15,18] The remaining studies were not analyzed because of their design (case reports and series < 3 cases), as they all reported a 100% positivity rate.[8,13,14,17] Among included studies, the individual positivity rate of MPXV DNA in semen specimens ranged from 54.17%[20] to as high as 90.63%.[6] The meta-analysis included a total number of 115 patients (82 showed positivity), revealing an overall positivity rate of MPXV in seminal specimens of 72.4% (95% CI: 55.7–84.5%; $I^2 = 60.62\%$) (Figure 2). Of note, the analyzed population is not reflective of the overall population included in these studies (Table 1) since only a minority of these populations were examined and analyzed.

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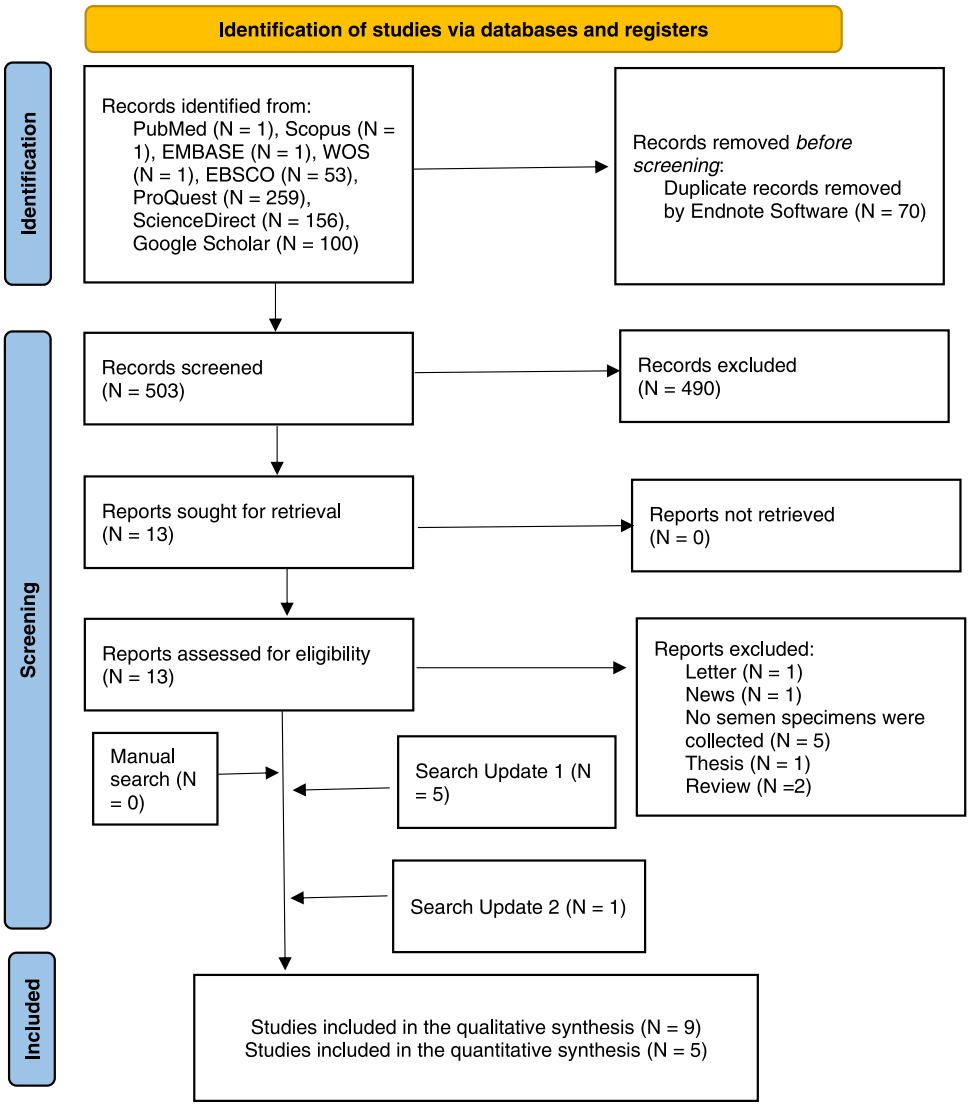

FIGURE 1 PRISMA flow diagram of the database search and screening processes. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

3.5 | Replication competence of MPXV detected in seminal specimens

Only two included studies, which included four MPX patients reported this outcome. Lapa et al.[13] showed the cytopathic effect after viral inoculation in the cell growth medium for 48-96 h after inoculating the viral specimen detected on the sixth day after the onset of symptoms in Vero E6 cells (ATCC). The authors furtherly indicated this in another patient with a quantification cycle value of 22.7.[23] On the other hand, Noe et al.[14] reported that using the same culture media, no cytopathic effects were found for MPXV particles obtained from seminal specimens in their two patients. This was also indicated for urine and plasma specimens, but not for particles obtained from pustules, which showed a cytopathogenic effect typical of orthopoxviruses 2 days after inoculation.

3.6 | The time interval between symptoms onset and the positivity of MPXV viral PCR in seminal fluid

Six studies[8,13–15,17,20] reported the time from symptom onset until the MPXV viral PCR results of the collected semen specimens of MPX-confirmed cases (Table 4). Although available data in this regard are scarce, the positivity of MPXV DNA PCR in the seminal fluid can be detected as early as within the first day of presentation[15,20] and remain positive up to 19 days following symptom onset.[13]

3.7 | The comparison of MPXV DNA positivity rate among different collected specimens

The comparison between different specimens in terms of MPXV positivity rate through viral detection with PCR is illustrated in

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TABLE 1 Baseline characteristics of studies examining monkeypox virus positivity in semen (*N* = 9)

Author/ YOP	Country	Study Design	Sample	Age	Male gender (%)	Presenting Symptoms/Complaints		History of Travel (N/T)	Previous Smallpox Vaccination (N/T)	Sexual Transmission (N/T)	Diagnostic Method
						Systemic (N)	Non-systemic (N)				
Antinori et al.[8]	Italy	Case series	4	30–39	100%	Fever (*N* = 1), Myalgia (*N* = 1)	Lymphadenopathy (*N* = 2)	(4/4)	(1/4)	MSM = (4/4)	RT-PCR from various samples (skin, anogenital lesions, serum, plasma, semen, feces, and nasopharynx), viral quantification cycle (Cq), and DNA sequencing
Mileto et al.[17]	Italy	Case report	1	33	100%	Fever, asthenia, malaise, and anorexia	Lymphadenopathy, faryngodynia, and sneezing	(1/1)	NR	MSM = (1/1)	RT-PCR from different sites (pharynx, skin lesions, anal ulcer, seminal fluid)
Noe et al.[14]	Germany	Case series	2	26–32	100%	Fever (*N* = 2), fatigue (*N* = 1), malaise (*N* = 1), back/ muscle/joint/anal pain (*N* = 1), and headache (*N* = 1)	Lymphadenopathy (*N* = 2), dysphagia (*N* = 1), and cough (*N* = 1)	(1/2)	NR	MSM = (1/2)	RT-PCR from different sites (urine-semen-blood-skin swap-oral lesion swap-Swab wrist pustule-swap head pustule), and DNA sequencing, viral quantification cycle (Cq)
Thornhill et al.[6]	Multi-country (*N* = 16)	Case series	528	38 (18–68)[a]	99.81%	Rash (95%), fever (62%), lethargy (41%), myalgia (31%), headache (27%), and low mood (10%)	Lymphadenopathy (56%), pharyngitis (21%), proctitis or anorectal pain (14%)	(147/528)	(49/528)	MSM = (509/528); Bisexual = (10/528)	RT-PCR from different sites (skin or anogenital lesion-nose or throat swab-Blood-Urine-Semen)
Lapa et al.[23]	Italy	Case report	1 (16)[b]	39, NR	100%	Fever (*N* = 1)	NR	(1/2)	(1/2)	MSM = (2/2)	RT-PCR from different sites (skin lesion-urine-semen-plasma samples)-MPXV DNA concentration was measured using quantification cycles

(Continues)

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TABLE 1 (Continued)

Author/ YOP	Country	Study Design	Sample	Age	Male gender (%)	Presenting Symptoms/Complaints		History of Travel (N/T)	Previous Smallpox Vaccination (N/T)	Sexual Transmission (N/T)	Diagnostic Method
						Systemic (N)	Non-systemic (N)				
Palich et al.[20]	France	Case series	50	34 (29–40)[c]	100%	Skin lesions (100%), Fever (52%), Exhaustion (54%), Headache (46%), Myalgia (38%)	Lymphadenopathy (54%), Rectitis (32%), Tonsilitis (16%), Cough (6%)	NR	(6/50)	MSM = (49/50)	RT-PCR from different sites (urine-semen-blood-skin swap-oral lesion swap-Swab wrist pustule-swap head pustule), and DNA sequencing, viral quantification cycle (Cq)
Peiró-Mestres et al.[15]	Spain	Case series	12	38.5 (32–52)[a]	100%	Fever (*N* = 4), Myalgia (*N* = 6), fatigue (*N* = 1), headache (*N* = 1), general malaise (*N* = 8)	Odynophagia (*N* = 2), proctitis (*N* = 3), and proctalgia (*N* = 1)	(4/12)	(4/12)	MSM = (12/12)	RT-PCR from different sites (saliva, rectal and nasopharyngeal swab, semen, urine and fecal samples)
Raccagni et al.[18]	Italy	Case series	36	31.5 (31.25–35.5)[c]	100%	NR	NR	NR	NR	MSM = (36/36)	RT-PCR from different sites (serum/plasma, seminal fluids, genitalia, skin, rectum, and urine)
Tan et al.[19]	Canada	Case report	1	40	100%	Fever, myalgia, and headache	Submandibular lymphadenopathy	NR	(0/1)	MSM = (1/1)	PCR from different sites (blood, pharynx, urine, and semen)

Abbreviations: MSM, men who have sex with other men; N, number; NR, not reported; RT-PCR, reverse-trascriptase polymerase chain reaction; T, total sample; YOP, year of publication.

[a]Median and range;

[b]16 patients were reported as a part of future non-published investigation by the same authors;

[c]Median and interquartile range.

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TABLE 2 Reported sexually-transmitted infections among MPX-confirmed cases in included studies

Author (YOP)	HIV (N/T)	HSV (N/T)	Chlamydia trachomatis (N/T)	Neisseria gonorrhea (N/T)	Syphilis (N/T)	Previous STIs	Concurrent STIs
Antinori et al.[8]	(2/4)	NR	NR	NR	(3/4)	NR	NR
Mileto et al.[17]	NR	NR	NR	NR	NR	NR	NR
Noe et al.[14]	(1/2)	NR	NR	NR	NR	NR	NR
Thornhill et al.[6]	(218/528)	(3/377)	(20/377)	(32/377)	(33/377)	NR	NR
Lapa et al.[23]	(2/2)	NR	NR	NR	NR	NR	NR
Palich et al.[20]	(22/50)	NR	NR	NR	NR	NR	NR
Peiró-Mestres et al.[15]	(4/12)	NR	(1/12)	(1/12)	(2/12)	NR	NR
Raccagni et al.[18]	(15/36)	NR	NR	NR	NR	(36/36)	(4/36)
Tan et al.[19]	(1/1)	(1/1)	NR	NR	(1/1)	NR	NR

Abbreviations: HAV, Hepatitis A virus; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HIV, Human immunodeficiency virus; HSV, Herpes simplex virus; N, Number of cases with STIs; NR, Not reported; STI, Sexually-transmitted infection; T, Total number of examined MPX-confirmed cases; YOP, Year of publication.

TABLE 3 The quality of included studies using the NIH tool for case series

Author (YOP)	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Overall Rating
Thornhill et al.[6]	Y	Y	NR	Y	NA	Y	Y	Y	Y	Good
Palich et al.[20]	Y	Y	NR	Y	NA	Y	Y	Y	Y	Good
Peiró-Mestres et al.[15]	Y	Y	NR	Y	NA	Y	Y	NA	Y	Good
Noe et al.[14]	Y	Y	NR	Y	NA	Y	Y	NA	Y	Good
Antinori et al.[8]	Y	Y	N	Y	NA	Y	Y	NA	Y	Good
Raccagni et al.[18]	Y	Y	N	Y	NA	Y	Y	NA	Y	Good

Note: Q1: Was the study question or objective clearly stated? Q2: Was the study population clearly and fully described, including a case definition? Q3: Were the cases consecutive? Q4: Were the subjects comparable? Q5: Was the intervention clearly described? Q6: Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants? Q7: Was the length of follow-up adequate? Q8: Were the statistical methods well-described? Q9: Were the results well-described?

Abbreviations: N, no; NA, not applicable; NIH, National Institute of Health; Y, yes; YOP, year of publication.

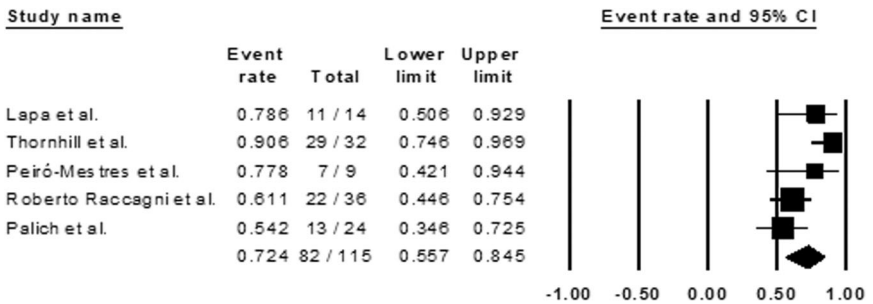

FIGURE 2 A forest plot showing the pooled rate of positive monkeypox viral PCR results in semen specimens of monkeypox-confirmed cases. PCR, polymerase chain reaction.

Table 5. Four studies[6,15,18,20] compared the positive rate of MPXV viral PCR tests among different specimens including salivary, oropharyngeal, nasopharyngeal, plasma, cutaneous, urinary, fecal, rectal, and genital samples. In our meta-analysis, compared to seminal samples (positivity rate of 72.4%), the positive rate of MPXV viral PCR was higher among skin samples (89%; 95% CI: 78.2%–94.8%; $I^2 = 0\%$), followed by anogenital/rectal samples (74.3%; 95% CI: 60.4%–84.5%; $I^2 = 44.99\%$). On the other hand, the positivity rate

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TABLE 4 Number of cases that showed positive monkeypox viral PCR result based on seminal analysis according to the day of assessment following symptom onset

Author (YOP)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 19
Antinori et al.[8]					(1/2)	(1/1)		(1/1)	(2/4)								
Mileto et al.[17]									N/A	(0/1)	N/A	N/A			N/A		N/A
Noe et al.[14]				(0/1)													
Lapa et al.[23]					(1/1)	(1/1)	(1/1)	N/A	N/A	N/A	N/A		N/A	(1/1)	(1/1)	N/A	(1/1)
Palich et al.[20]	(13/24)													(9/11)			
Peiró-Mestres et al.[15]	(1/12)	(0/12)	(0/12)	(2/11)	(0/11)	(1/12)	(2/12)	(1/11)	(0/12)	(1/12)	(0/10)	(0/11)	(1/11)	(1/12)	N/A	(1/12)	

Note: Data are presented as (the number of cases with positive seminal fluid for monkeypox virus/total number of assessed patients). Blank cells mean that semen specimen were not collected that day.
Abbreviations: YOP, Year of publication; N/A, Not assessed during that day.

was lower in nasopharyngeal (62.4%; 95% CI: 20.4%–91.5%; $I^2 = 95.88\%$), urine (21.1%; 95% CI: 4.3%–61.1%; $I^2 = 95.24\%$), and blood/plasma (14.3%; 95% CI: 11.3%–18.1%; $I^2 = 97.52\%$) samples. Moreover, the prevalence rates of MPXV viral PCR in saliva 100%, and feces were 100% and 66.66%, respectively. However, we could not conduct a meta-analysis because reported data were limited to an individual report per specimen site (Table 5).

4 | DISCUSSION

The main aim of the current study is to provide more insight into MPXV transmission through seminal fluids of infected individuals. Our findings indicate the high prevalence of positive MPXV DNA in the seminal fluid samples obtained from infected patients. This rate was found to be the highest followed by positive rates in skin and anogenital/rectal samples of MPX cases.

Viral transmission, replication, and infectivity through semen is a vital topic in understanding the epidemiology of the current multi-country outbreak. Although our study shows that MPXV is transmitted through semen at a high rate, the replication and infectivity of the virus through semen remains controversial.[24] The study by Lapa et al.[13] was the only one to report that MPXV detected in the semen samples of their two MPX patients within the acute phase of the infection might contain a replication-competent virus. The replication competency in this study was indicated by the cytopathic effect after viral inoculation in the cell growth medium for 48–96 h. The authors furtherly suggested that MPXV might have a genital reservoir because of the prolonged viral shedding, even at low viral copies, in seminal samples. On the other hand, Noe et al.[14] showed that on growing the MPXV seminal samples of their two MPX patients in cell culture (VeroE6), no growth was observable, unlike pustule materials, which showed a cytopathogenic effect typical of orthopoxviruses. MPXV DNA presence in the seminal fluids might be due to local genital replication or passive diffusion from urine, blood, or genital lesions.[25] However, the exact mechanism of this event remains controversial in the literature. Although Lapa et al.[13] reported that cross-contamination from other sources (blood and urine) is unlikely (due to the absence of viral DNA in their specimens), our findings show the high prevalence of positive MPXV DNA from different specimens, like the rectal, urinary, skin, and fecal ones, which might attribute to cross-contamination in some studies. Moreover, we found that only 14.3% of the blood/plasma specimens were positive for MPXV DNA. However, MPXV viremia was established in previous reports,[14,26] which might also be another reason for MPXV presence in the seminal fluids of MPX patients.

MPXV transmission through seminal fluids depends on the ability of the virus to replicate within this media. Accordingly, although the current study demonstrated a high prevalence rate of MPXV in the tested seminal samples, this rate does not indicate viral infectivity since we could not provide more evidence regarding viral replication competence.[6] Therefore, the infectivity of seminal MPXV remains controversial and needs further investigations.[24] It should be noted

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TABLE 5 Monkeypox viral PCR positivity according to the site of the collected specimen

Site of positive MPX viral PCR	Studies (*N*)	Patients (Event/Total)	Pooled ES [95% CI]	Model/Method	I^2
Saliva[a]					
	1	12	100%	N/A	N/A
Nasopharynx[a]					
	3	184/587	62.4% [20.4%–91.5%]	Random effects	95.88%
Blood/Plasma					
	3	72/609	14.3% [11.3%–18.1%]	Random effects	97.52%
Skin[b,a]					
	2	56/62	89% [78.2%–94.8%]	Fixed effects	0%
Urine					
	4	40/617	21.1% [4.3%–61.1%]	Random effects	95.24%
Feces					
	1	8/12	66.66%	N/A	
Anogenital/Rectal[b,a]					
	2	41/54	74.3% [60.4%–84.5%]	Fixed effects	44.99%
Semen[c]					
	5	82/115	72.4% [55.7%–84.5%]	Random effects	60.62%

Abbreviations: CI, confidence interval; ES, effect size; I^2, heterogeneity measure; MPX, monkeypox; N/A, not applicable; PCR, polymerase chain reaction.

[a]The study by Raccagni et al. was excluded from the pooled analysis of the skin, anogenital, saliva, and nasopharynx samples since the authors provided a combined rate for these samples (36/36) that could not be separated;

[b]The study by Thornhill et al. was excluded from the pooled analysis of the skin and anogenital samples since the authors provided a combined rate for these samples (512/528) that could not be separated;

[c]We included the preliminary non-published results by Lapa et al., which reported that MPXV was present in 11/14 seminal specimens.

that Noe et al.[14] furtherly demonstrated that skin swabs had the highest viral concentrations in their patients. This strengthens the fact that contact transmission through MPXV-related lesions is another main route of infection. This does not exclude the role of sexual activities in spreading the disease. In fact, if the infection is not transmitted through seminal fluids, it might also spread through other ways, like contacting skin lesions. Compared with other infections-causing pandemics, previous studies also indicated that COVID-19 patients have SARS-CoV-2 in their seminal and feco-anal specimens.[27] However, the rates reported for the presence of SARS-CoV-2 viral RNA detection in semen samples are remarkably lower than the currently estimated rate for MPXV DNA in the current study.

Importantly, our study has some limitations. First, the current number of relevant studies and the sample size per study are small. Accordingly, the currently reported rates might not be the best estimation since pooling is based on a low number of patients, and some data were obtained from case series studies. Moreover, data regarding the prevalence of positive MPXV DNA in seminal specimens obtained from Lapa et al.[13] is preliminary, which might also limit the current estimation due to improper design. However, a random effect model was used whenever we encountered heterogeneity among the pooled outcomes. Second, we could estimate the prevalence rates of positive PCR samples from different specimens. However, we could not determine the infectivity of MPXV detected in urine samples, although the prevalence rate is high among them, because of lacking proper data investigating the replication-competence of the virus in this medium. Although the high prevalence rate of positive MPXV DNA in seminal samples potentially excludes cross-contamination, the current evidence is not definite and needs further strengthening.

5 | CONCLUSION

This is the first study to provide cumulative evidence regarding the prevalence of positive MPXV DNA in the seminal fluids of MPX patients. Our findings indicate the high prevalence of positive MPXV DNA in these specimens. However, the infectivity of these specimens is yet to be determined due to current insufficient evidence regarding viral replication competence.

AUTHOR CONTRIBUTIONS

Study concept and design: Abdullah Reda and Abdelaziz Abdelaal, Acquisition of data: Abdullah Reda, Abdelaziz Abdelaal, Aml M. Brakat, Basant Ismail Lashin, Basel Abdelazeem, and Moustafa

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Abouelkheir, analysis and interpretation of data: Abdullah Reda and Abdelaziz Abdelaal, Manuscript writing: all members, Supervision: Alfonso J. Rodriguez-Morales and Ranjit Sah, revision and approval: all members.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data will be provided upon request from Dr. Abdullah Reda (E-mail: Abdullahreda77@azhar.edu.eg).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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