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REVIEW

Detection of Mpox Virus in Seminal Fluids: Implications for Sexual Transmission

Angelo Roberto Raccagni[1], Antonella Castagna[1,2], Silvia Nozza[1,2]

[1]*Vita-Salute San Raffaele University, Milan, Italy;*
[2]*Infectious Diseases Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy*

SUMMARY

The 2022 outbreak of the human mpox virus, formerly known as monkeypox, raised global health concerns with widespread transmission across multiple countries. Sexual transmission emerged as a significant mode of spread, particularly among high-risk groups like MSM and PLWH. This manuscript focuses on the implications of seminal fluids in the transmission of mpox. The virus has been detected in various bodily fluids, including semen, indicating the potential for sexual transmission. Studies have reported high positivity rates of mpox DNA in seminal fluids. Despite some concern about possible contamination due to genital lesions, the presence of replication-competent virus in seminal fluids has been confirmed and mpox virus was also detected in this specimen among people who engaged only in receptive sexual intercourse. Antiviral treatment with tecovirimat showed efficacy in reducing viral presence in semen with detection of the antiviral in this specimen. Virus clearance from semen is relatively rapid and parallels healing from infection, with no reported cases of seminal fluid relapses. The WHO recommendation to avoid condomless intercourse for 12 weeks after clinical healing still appears prudent. Continued research and surveillance are essential to understand viral dynamics and develop effective prevention measures to combat the spread of mpox through sexual transmission and protect key-populations.

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Introduction: Mpox Epidemiology

In May 2022, a significant outbreak of the human mpox virus (formerly known as monkeypox) caused by the mpox virus emerged, spreading to Europe and North America, and prompting the World Health Organization (WHO) to declare it a public health emergency of international concern (PHEIC) (Thornhill JP, 2022; Mitjà O, 2022). The outbreak raised global alarms as the total number of laboratory-confirmed mpox cases reached a staggering 87,929 across 111 countries, resulting in 146 reported deaths (Mitjà O, 2023). The most affected regions, as reported by the WHO, were the Region of the Americas and the European Region. Although cases in 2023 saw a significant decrease, sporadic infections continue to be observed, and it remains crucial to closely monitor the dissemination of the virus to avoid any resurgence of cases.

Key words:
Mpox, Seminal Fluids, Semen.

Corresponding author:
Angelo Roberto Raccagni, MD
E-mail: raccagni.angelo@hsr.it

Mpox virus: Pathogenesis and Clinical Presentation

The mpox virus is an enveloped double-stranded DNA virus classified under the Orthopoxvirus genus, belonging to the Poxviridae family. It displays two distinct strains: clade one (I), which historically has been responsible for disease outbreaks in the Congo Basin and Central Africa, and clade two (II), first isolated in West Africa (Yinka-Ogunleye A *et al.*, 2019; Mitjà O, 2022). Clade II further splits into two subclades, IIa and IIb, with IIb exhibiting lower virulence. Global notifications and evidence suggest that the current mpox epidemic likely originated from the West Africa clade (Yinka-Ogunleye A *et al.*, 2019; Ogoina D, 2019). Historically, mpox cases were primarily concentrated in the WHO African Region, although sporadic cases were reported worldwide among individuals returning from travels (Yinka-Ogunleye A *et al.*, 2019). The first outbreak of mpox in the Western hemisphere occurred in the United States in 2003, followed by isolated cases in non-endemic countries often linked to travel (Thornhill JP, 2022). The current global outbreak began with the identification of the first mpox case in the United Kingdom in mid-May 2022, and since then additional cases have been reported worldwide (Mileto D, 2022). Community transmission has

been observed, being unrelated to recent travel or close contact with known mpox cases. The lack of clarity surrounding the natural history of mpox necessitates further research to identify potential reservoirs and understand the mechanisms of how the virus circulates in the environment and the related aspects of animal-to-human transmission. The incubation period for mpox varies, ranging from 4 to 21 days. Exposure with mucosal involvement results in a shorter incubation period compared to transmission routes involving droplets. The pathogenesis of mpox following skin inoculation appears similar to that of smallpox and other Orthopoxviruses, involving viral replication in the skin and lymphatic system. The formation of skin lesions undergoes stages, starting with vesicular, followed by pustule and ultimately crust formation. The majority of mpox cases experience mild to moderate symptoms and recover with supportive care (Thornhill JP, 2022; Fink DL, 2023; Tarín-Vicente EJ, 2022). However, severe cases with higher fatality rates have been reported, particularly among immunosuppressed patients, young adults, and children. Disfiguring scarring of the skin is a common sequela following infection clearance (Angelo KM, 2023). Vaccination has emerged as a crucial tool in controlling mpox outbreaks, with the MVA-BN vaccine recommended for high-risk individuals, including healthcare personnel with potential direct exposure, high-risk MSM, and others at risk of repeated exposure. Vaccination can be administered as a pre-exposure prophylaxis (PPV) for those at risk of mpox or as a post-exposure preventive vaccination (PEPV) for close contacts of cases to prevent disease onset or severity (Candela C, 2023).

Tracing Transmission Routes: From Animals to Humans and Human to Human

The mpox virus exhibits the capability of transmission both from animals to humans and from human to human. Historical outbreaks in the 1970s and 1980s were primarily associated with animal-to-human transmission, while more recent outbreaks, including the 2022 epidemic, are linked to human-to-human transmission. There is also evidence to suggest that human-to-animal transmission could be a possible route of infection. Transmission from infected animals to humans occurs through various means, including bites, scratches, exposure to animal blood or bodily fluids, prolonged close contact, and consumption of undercooked animal meat (Pan D, 2023). Additionally, human-to-human transmission is possible through diverse routes, with the primary mode being close contact with infectious material from cutaneous and/or mucosal lesions and bodily fluids. This includes inhalation of respiratory droplets after prolonged face-to-face contact, contact with bodily fluids or infectious lesions, contamination of surfaces or objects (fomites), and vertical transmission from mother to child (Pan D, 2023). Healthcare professionals are also at risk, as infections have been reported after needle stick injuries. Sexual activity emerged as the most significant risk factor during the 2022 outbreak, with cases linked to the International Pride Parade on the Spanish island of Gran Canarias leading to transmission chains in several European countries. Risky sexual behaviours, such as having multiple partners, attending sex parties or social gatherings, and using recreational drugs during sex, were strongly associated with infection (León-Figueroa DA. 2023; Hornuss D, 2023). Men who have sex with men (MSM), HIV pre-exposure prophylaxis (PrEP) users, and people living with HIV (PLWH) were disproportionately affected, leading to the majority of diagnoses being made at sexually transmitted infections (STIs) services or HIV clinics (Thornhill JP, 2022; Fink DL, 2023). Locally acquired community transmission was prevalent across affected countries, with concomitant STIs commonly reported, including syphilis, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae* (Candela C, 2023). The WHO recommended avoiding sexual contact among mpox cases during the 21-day monitoring period, regardless of symptoms. However, mpox cases were also described among women and non-binary individuals, highlighting that the disease can affect anyone (Thornhill JP, 2022).

Detection of Mpox in Seminal Fluids: Implications for Sexual Transmission

Replication-competent mpox virus has been isolated primarily from different clinical samples, including cutaneous lesions and oropharyngeal swabs. Mpox DNA has also been detected in various specimens, including skin, oropharyngeal mucosa, semen, urine, faeces, and saliva (Suñer C, 2023; Peiró-Mestres A, 2022; Candela C, 2023; Colavita F, 2023; Palich R, 2023; Noe S, 2022). More importantly, the presence of mpox DNA and isolation of replication-competent virus by means of viral cultures have also been detected in semen samples, corroborating the hypothesis of sexual transmission (Reda A, 2023; Barboza JJ, 2023). Understanding the role of semen in viral transmission is crucial for devising effective prevention strategies and controlling the spread of sexually transmitted infections.

Various viruses have been detected in seminal fluids, emphasizing their potential for sexual transmission. One notable example is HIV, which can be present in semen, making unprotected sexual intercourse the most significant mode of transmission (Muciaccia B, 2014). Another virus found in seminal fluids is the Zika virus, primarily transmitted through mosquito bites, but also posing a risk of sexual transmission, particularly from infected males to their partners (Mansuy JM, 2016). Certain types of Human Papillomavirus (HPV), especially high-risk strains, have also been identified in seminal fluids. Cytomegalovirus

(CMV), a herpesvirus, is another example found in various body fluids, including semen, and can be transmitted sexually. Both Herpes Simplex Virus (HSV) types 1 and 2, causing oral and genital herpes, have been found in seminal fluids (Jin F, 2006). Similarly, the presence of mpox virus in seminal fluids raises concerns about the role of sexual activity in driving the spread of mpox. Since the early phase of the current mpox virus outbreak, viral detection in seminal fluids has emerged (Lapa D, 2022; Raccagni AR, 2022). Overall, the positivity of mpox in seminal fluids has been reported to vary between 54% and 90%, with heterogeneous findings when comparing different populations (Suñer C, 2023; Peiró-Mestres A, 2022; Raccagni AR, 2022; Antinori A, 2022; Colavita F, 2023). However, positivity rates were found to be high in most case series and cohorts. An early metanalysis estimated the positivity rate to be 72.4% (Reda A, 2023), although the growing evidence on mpox transmission and existing cohorts will likely add significant knowledge to the available data.

For instance, in an Italian cohort of 36 MSM, 61% had positive seminal fluids with a median cycle threshold of 34 (interquartile range, 29-36.5) (Raccagni AR, 2022). Some authors correctly raised concern regarding the possible detection of mpox virus in seminal fluids due to contamination from genital lesions. However, positive seminal fluids were also detected among people without genital lesions or who did not report any insertive sexual intercourse (Raccagni AR, 2022). Thornill *et al.* (2022) reported very frequent virus detection among 29 out of 32 tested individuals in the earliest international collaborations, showing that close monitoring and investigation on this topic was present from the very beginning of this outbreak. Moreover, virus was cultured from different specimens, including seminal fluids specimens, highly corroborating that during sexual transmission not only close skin contact, but also viral spread by means of bodily fluids, can likely amplify transmission (Lapa D, 2022; Colavita F, 2022). Positive cultures showed that virus capable of replicating and infecting was also present at the genital site, which favoured the WHO recommendation to avoid condomless sexual intercourse following clinical recovery of skin from mpox. Viral dynamics of mpox has been found to be highly heterogeneous among infected people (Tarín-Vicente EJ, 2022; Suñer C, 2023). For instance, cases of late positivization of samples which first tested negative at time of mpox diagnosis were reported, including seminal fluids. People who did not show presence of mpox virus at specific sites at the beginning of infection were later found, during follow-up, to start spreading virus from seminal fluids or from the oropharynx (Raccagni AR, 2023). Reassuringly, a prospective cohort study found that 33/49 (67%) semen samples tested positive for mpox virus and that the days of virus detection in seminal fluids was only 13 (interquartile range, 9-18). For 90% of cases, the time from symptom onset to viral clearance was 41 days (95% CI 34-47) in skin lesions and 39 days (27-56) in semen (Suñer C, 2023). In this study, virus isolation by means of culture in semen samples was found to be uncommon. All in all, various evidence regarding the possibility to culture viable and replication-competent mpox virus is currently available; however, only a few cases of replication competent mpox virus in seminal fluids have been reported to date.

Treatment, Persistence, and the Future

According to international guidelines, Mpox virus can be treated with antivirals, including tecovirimat, cidofovir and brincidofovir, particularly in severe cases (Mitjà O, 2022). Recently, Tempestilli *et al.* (2023) reported that tecovirimat concentrations were detectable in seminal fluids of people diagnosed with mpox who received antiviral therapy, with sustained negativization of the virus in this specimen. Antiviral concentrations were detectable in this body fluid and in plasma, demonstrating drug penetration in the genital tract of men diagnosed with mpox. The observed concentrations exceeded the 50% inhibitory concentration of mpox in vitro. This underscores the importance of antiviral treatment not only in managing the disease but also in reducing virus transmission and enhancing infection control measures (Tempestilli, 2023).

Regarding virus persistence, some authors hypothesized the existence of a genital reservoir. Reassuringly, recent data support that once infection is cleared from seminal fluids individuals do not test positive for mpox over long-term follow-up, indicating a lack of viral relapses at the genital site. However, infection persistence and cases of suspected re-infection have been described to date, warranting further investigation into the viral dynamics of this old yet new infection (Musumeci S, 2023; Raccagni AR, 2023; Golden J, 2023). Moreover, in a prospective cohort study, the overall risk of transmission through seminal fluid was estimated to be probably low and the time to clearance of replication-competent virus appeared shorter than the duration of viral detection by PCR (Suñer C, 2023).

CONCLUSION

In conclusion, the transmission of mpox virus is complex and involves multiple routes between humans, with sexual exposure being predominant during the 2022 outbreak. Understanding the various modes of transmission and the viral dynamics in seminal fluids is crucial to control and prevent further outbreaks of monkeypox. Although mpox DNA was found very frequently in seminal fluids of infected individuals, it was not always possible to demonstrate the presence of replicating competent virus. Infection clearance

from seminal fluids was found to be rapid and parallel to healing from infection, and no cases of relapses in semen have been reported to date. Nevertheless, the WHO paradigm of avoiding condomless intercourse for 12 weeks following clinical healing from mpox infection appears to have been correctly cautious and important to avoid amplification of transmission. Although sexual transmission has been well-demonstrated to be the primarily mode of transmission of mpox during the current outbreak, the debate regarding whether mpox should be categorized as a "sexually transmitted infection" is still open. For instance, in order to avoid stigmatization and keep the focus on the fact that infection involves different modes of transmission, such occupational and maternal, we suggest referring to mpox as a "sexually transmissible infection." This reflects the strong link with high-risk sexual behaviours in mpox transmission dynamics, considering other important routes as well, which in the future might also be predominant (Allan-Blitz LT, 2023; Hazra A, 2023; Rodriguez-Morales A, 2022). We believe that thanks to the collective efforts of international researchers, which included both retrospective and prospective cohorts from the very beginning of the outbreak, mpox transmission dynamics and their link to sexual behaviours have been well characterized. Nevertheless, further studies on mpox infectiousness are needed, especially given the recently demonstrated cases of asymptomatic infections and mpox re-infection (Hazra A, 2023; Moschese D, 2023). Continued research and surveillance are essential to improve prevention strategies, treatment options, and infection control measures, ultimately mitigating the impact of this emerging infectious disease on public health. Vigilance and collaborative efforts among the global health community are necessary to effectively combat the spread of mpox and protect people belonging to key populations.

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Transparency declarations

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