International Journal of Infectious Diseases 160 (2025) 108041

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Assessment of seroprevalence of Mpox virus infection among sex workers in Chiang Mai, Thailand

Muhammad Umer[1,2], Kriangkrai Chawansuntati[2], Jiraprapa Wipasa[2], Kanyaruck Jindaphun[2], Kamonporn Kotemul[2], Yee Mon Thant[3,4], Nicole Ngo-Giang-Huong[4,5,6], Woottichai Khamduang[4,5,7], Sayamon Hongjaisee[1,2,*]

[1] School of Health Sciences Research, Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, Thailand
[2] Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, Thailand
[3] Office of Research Administration, Chiang Mai University, Chiang Mai, Thailand
[4] LUCENT international collaboration, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand
[5] LMI PRESTO, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand
[6] Maladies Infectieuses et Vecteurs: Écologie, Génétique, Évolution et Contrôle (MIVEGEC), Agropolis University Montpellier, Centre National de la Recherche Scientifique (CNRS), Institut de Recherche Pour le Développement (IRD), Montpellier, France
[7] Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand

ARTICLE INFO

Article history:
Received 4 August 2025
Revised 29 August 2025
Accepted 30 August 2025

Keywords:
Mpox virus
Seroprevalence
Sex workers
In-house ELISA
E8L antigen
Thailand

ABSTRACT

Objectives: The 2022 global Mpox outbreak revealed major surveillance gaps, particularly among marginalized populations such as sex workers, whose role in undetected MPXV transmission remains poorly understood. This study aims to assess the seroprevalence of MPXV IgG antibodies and identify associated risk factors among sex workers in Chiang Mai, Thailand.

Methods: In this cross-sectional study, 262 sex workers recruited from March to December 2022 were tested for prior MPXV exposure using an in-house indirect ELISA targeting the recombinant MPXV E8L antigen. Logistic regression models were used to identify demographic and behavioral factors associated with seropositivity.

Results: MPXV IgG seroprevalence was 4.6% (12/262). Seropositivity was significantly higher among males than females (5.2% vs 4.0%, $P = 0.011$). In multivariable analysis, male gender (OR = 3.23, 95% CI: 1.10-9.47, $P = 0.032$), blood transfusion history (OR = 4.40, 95% CI: 1.35-14.25, $P = 0.013$) and sharing sharp objects with others (OR = 4.55, 95% CI: 1.12-18.48, $P = 0.034$) were significantly associated with Mpox seropositivity.

Conclusion: Our findings reveal undetected MPXV circulation among Chiang Mai sex workers. The identified risk factors suggest overlapping parenteral and sexual vulnerabilities, emphasizing the need for integrated surveillance and targeted interventions.

Introduction

In July 2022, the World Health Organization (WHO) declared the multi-country Mpox outbreak (formerly monkeypox) a Public Health Emergency of International Concern (PHEIC) due to its rapid spread through sexual transmission in countries where the virus had not previously been reported [1]. The PHEIC designation was lifted in May 2023 following a sustained global decline in Mpox cases [2]. Multiple outbreaks involving various Mpox clades have occurred across different countries, each characterized by distinct transmission modes and varying levels of risk.

Mpox, caused by Mpox virus (MPXV), is a zoonotic disease belonging to the *Orthopoxvirus* genus of the *Poxviridae* family. Human Mpox typically presents as a self-limiting febrile illness characterized by high fever, lymphadenopathy, fatigue, and a vesiculopustular rash. However, recent outbreaks have demonstrated atypical clinical features, including frequent anogenital and mucosal involvement, such as proctitis and oropharyngeal lesions, with severe complications observed in immunocompromised individuals [3]. Transmission primarily occurs through close physical contact with infected individuals, including direct contact with skin lesions, body fluids, and respiratory droplets. This transmission pat-

* Corresponding author. Research Institute for Health Sciences, Chiang Mai University, 110, Intawaroros Rd., Sripoom, Muang, Chaingmai 50200 Thailand.
E-mail address: sayamon.ho@cmu.ac.th (S. Hongjaisee).

https://doi.org/10.1016/j.ijid.2025.108041

tern has been particularly evident in recent outbreaks, which have disproportionately affected men who have sex with men (MSM) and immunocompromised individuals [4].

During the 2022 outbreak, in Spain, 99% of Mpox cases were male, with 93% identifying as MSM, and transmission was primarily linked to close physical contact during sexual activity [5]. In the United States, most confirmed cases of Mpox have been among gay, bisexual, queer, and other MSM, with New York State reporting the most [6]. Notably, some individuals with Mpox infection may remain asymptomatic or exhibit no characteristic lesions, or they may clear the virus without clinical recognition. Consequently, many studies have focused on seroprevalence surveys to identify prior exposure by detecting Mpox-specific antibodies, providing a more accurate estimate of the true burden of infection.

A cross-sectional seroprevalence study among MSM in Berlin conducted in 2023 showed that 7.4% had a previously diagnosed Mpox infection, while an additional 91 undiagnosed cases were identified serologically; higher numbers of condomless anal sex partners were strongly associated with seropositivity, highlighting silent transmission potential in sexual networks [7]. A study conducted in South Florida identified 5.1% seropositivity in a cohort including people living with HIV (PLWH) without reported vaccination or prior Mpox diagnosis [8]. Another study in Rome reported 7.4% seroprevalence among PLWH without prior vaccination or Mpox diagnosis, indicating a meaningful burden of asymptomatic infections even beyond traditional high-risk groups such as MSM, with seropositivity seen across diverse genders and ethnicities [9].

Given the global stigma-related challenges in Mpox detection, particularly among marginalized populations, tailored surveillance approaches have emerged. Recent studies from Thailand and Southeast Asia highlight underrecognized Mpox transmission in these groups. MPXV DNA was detected in non-sewered wastewater in Bangkok as early as June 2022, and in multiple Southeast Asian countries, signaling ongoing local transmission despite limited case reporting [10–12]. Additionally, community-based surveillance using discarded condoms successfully identified Mpox and STIs among sex workers in Thailand, underscoring the value of venue-based screening for reaching high-risk, underserved populations [13]. These findings support the urgent need for targeted surveillance in settings with social and structural barriers to diagnosis.

However, to date, no seroprevalence studies have focused on sex workers, despite their clear vulnerability to Mpox infection due to frequent close contact with multiple partners, inconsistent condom use, and structural barriers such as stigma, legal restrictions, and limited healthcare access underscoring a critical gap in understanding the burden of asymptomatic or undiagnosed Mpox in this high-risk population.

To address this gap, we conducted a seroprevalence study among sex workers in Chiang Mai, Thailand during the 2022 outbreak, to estimate the prevalence of prior MPXV exposure and to identify risk factors associated with MPXV seropositivity in this population.

Materials and methods

Study population

This cross-sectional study utilized stored plasma samples and participant data from a cohort of sex workers in Chiang Mai, Thailand, recruited between March and December 2022, previously described by Hongjaisee et al. [14]. The original study enrolled adults (≥18 y) of Thai or migrant background, who were sexually active within the past 12 mo and currently working in entertainment venues or as freelance sex workers in Chiang Mai. The information on socio-demographic characteristics, sexual behaviors, and health history was collected via structured interviewer-administered questionnaires using the REDCap® platform.

Preparation of indirect ELISA assay

Recombinant full-length E8L envelope protein of Mpox virus (Cat. No. 40890-V08B; Sino Biological), expressed in baculovirus-insect cells, was used as the capture antigen. Anti-MPXV plasma, derived from pooled plasma of 100 individuals recovered from Mpox infection in the Democratic Republic of Congo (NIBSC code: 22/218; National Institute for Biological Standards and Control), was used as the positive control.

A 96-well flat-bottom Nunc MaxiSorp® plate (Thermo Fisher Scientific) was coated with 1% skimmed milk in half of the wells and E8L antigen (1 μg/mL) in the other half, both prepared in carbonate coating buffer (Na_2CO_3, $NaHCO_3$, ultra-pure water, pH 9.6). The plate was incubated overnight at 4 °C in a humidified chamber. After incubation, wells were washed four times with washing buffer (PBS + 0.05% Tween 20, pH 7.4), excess liquid was removed by tapping the plate on absorbent paper, and wells were blocked with 200 μL/well blocking buffer (PBS + 1% skimmed milk, pH 7.4) at 37 °C for 1 h. The blocking buffer was then discarded without further washing.

Indirect ELISA assay for detection of anti-Mpox

An in-house indirect ELISA was performed to detect human IgG antibodies against the MPXV E8L antigen. Test samples, positive control, and negative control sera were diluted 1:50 in diluent buffer (blocking buffer with 0.05% Tween 20) and added in duplicate (50 μL/well) to both E8L antigen-coated and skimmed milk-coated wells. The skimmed milk-coated wells served as background controls to account for nonspecific binding or background noise. Plates were incubated at 37 °C for 1 h, followed by four washes with washing buffer. Subsequently, 50 μL/well of 1:2000 diluted goat anti-human IgG HRP-conjugated antibody in diluent buffer was added and incubated at 37 °C for 1 h. A second set of four washes was then performed. After each wash step, the plate was tapped on absorbent paper to remove residual liquid. Next, 50 μL/well of TMB substrate was added and incubated in the dark for 15-20 min. The reaction was stopped with 25 μL/well of 0.2 M H_2SO_4, and optical density (OD) was measured at 450 nm using a CLARIOstar® Plus microplate reader (BMG Labtech). Final OD values were calculated by subtracting the background signal (OD of skimmed milk-coated wells) from the corresponding E8L antigen-coated wells. A schematic flowchart summarizing the ELISA procedure is presented in Figure 1.

Determination of the cut-off value

The assay cut-off was defined as three times OD of the negative control serum (cut-off = $OD_{neg} \times 3$). To ensure assay validity, a cut-off value >0.250 was required; runs below this threshold were considered invalid and repeated. The signal-to-cut-off (S/CO) ratio was calculated by dividing the OD of each sample by the cut-off OD from the corresponding run. Samples with an S/CO ratio >1.1 were classified as positive, those with a ratio <0.9 as negative, and ratios between 0.9 and 1.1 were considered borderline.

Data analysis

Descriptive statistics for the study population were previously reported by Hongjaisee et al. [14] and are not reanalyzed here. Categorical variables were summarized as frequencies and percentages, while continuous variables were reported as medians with

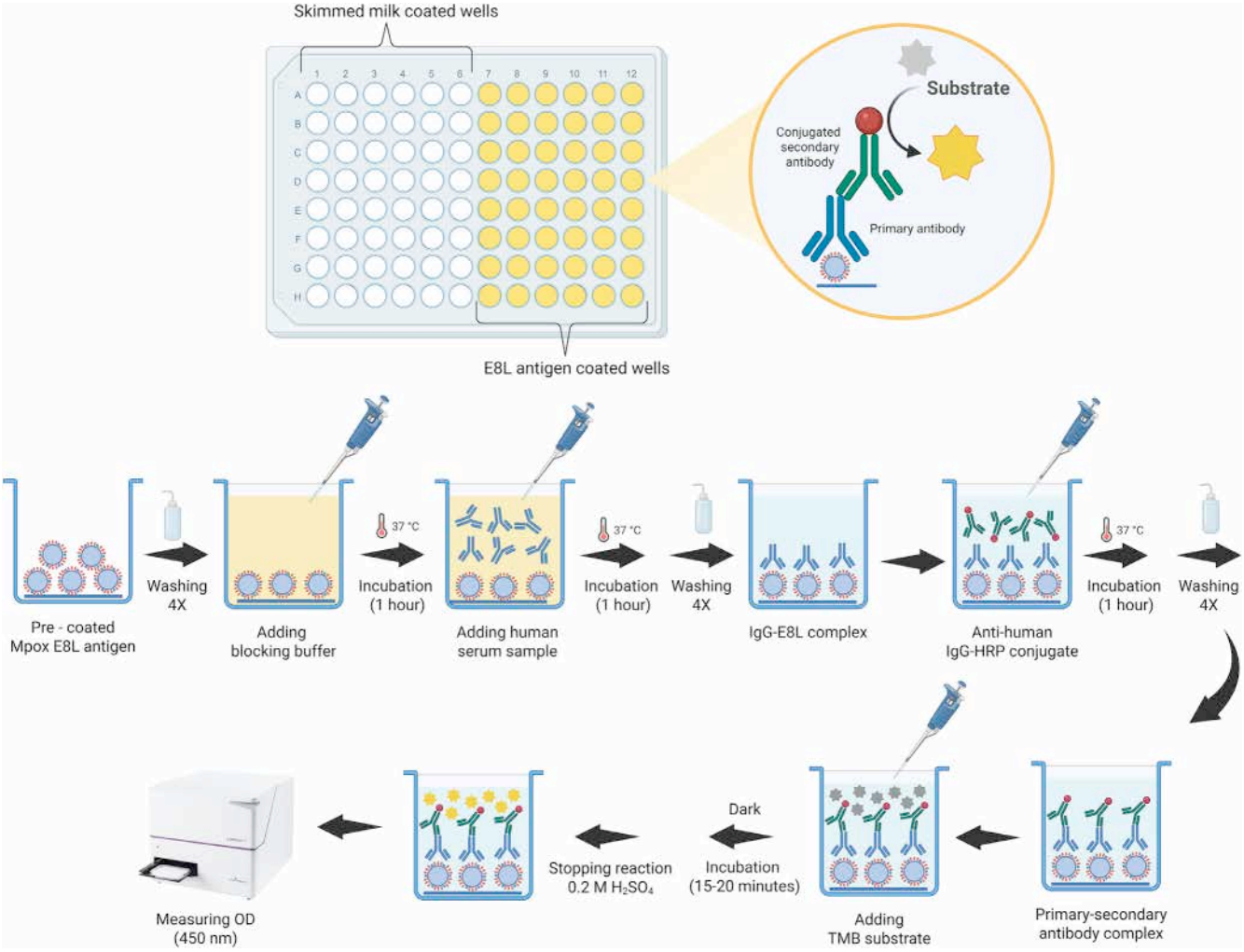

Figure 1. Schematic flowchart of Anti-Mpox IgG in-house indirect ELISA.

interquartile ranges (IQRs). For the current analysis, associations between potential risk factors and Mpox seropositivity were assessed using univariable and multivariable logistic regression analyses. Variables with a *P*-value < 0.250 in univariable analysis were included in multivariable logistic regression analysis. A forward stepwise selection approach was used, beginning with the variable most strongly associated with the outcome. Odds ratios (OR) and 95% confidence intervals (CI) were reported to indicate the strength of association. The *P*-value < 0.05 was considered statistically significant. All analyses were performed using Stata version 16.0 (StataCorp, College Station).

Results

Characteristics of the study population

The study population, described in detail by Hongjaisee et al. [14], comprising 262 sex workers of both sexes, with male accounting for 51.9%. Their median age was 31 y (IQR: 25-38) overall, with men younger (27 y) and women older (35.5 y) on average. Socio-demographic and behavioral characteristics were comparable to those reported in the prior study, which analyzed a total of 264 participants from the same recruitment cohort. Two samples were excluded from the present study due to insufficient volume.

Seroprevalence of Mpox

Among the 262 sex workers tested, the overall Mpox seroprevalence was 4.6%, as summarized in Table 1. Seropositivity was slightly higher in males at 5.2% compared to 4.0% in females. Additionally, 3.4% of participants had borderline serological results, all of whom were male, corresponding to 6.6% among men. The majority of participants, 92%, tested negative for Mpox antibodies, including 88.2% of males and 96.0% of females. The overall difference in serological outcome between sexes was statistically significant ($P = 0.011$).

Factors associated with Mpox seroprevalence

Factors associated with Mpox IgG seropositivity among sex workers were analyzed using two interpretive definitions: one considering borderline results as positive, and another considering them as negative. Univariable and multivariable logistic regression analyses were performed to identify variables potentially associated with Mpox seropositivity, as detailed in Table 2. Variables with $P < 0.250$ in univariable analysis were included in the multivariable model.

When borderline results were treated as positive, multivariable analysis revealed significant associations between Mpox seropositivity and a history of blood transfusion (OR = 4.40, 95%

Table 1
Seroprevalence of Mpox among sex workers in Chiang Mai, Thailand ($N = 262$).

Mpox seroprevalence	Total ($N = 262$)		Male ($N = 136$)		Female ($N = 126$)		*P*-value*
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	
Positive	12 (4.6)	2.4-7.9	7 (5.2)	2.1-10.3	5 (4.0)	1.3-9.0	**0.011**
Borderline	9 (3.4)	1.6-6.4	9 (6.6)	3.1-12.2	0	0	
Negative	241 (92.0)	88.0-95.0	120 (88.2)	81.6-93.1	121 (96.0)	91.0-98.7	

Bold values represent $p < 0.05$, considered statistically significant.
* *P*-value refers to the comparison of Mpox seroprevalence between male and female participants.

CI: 1.35-14.25, $P = 0.013$) as well as gender (male) (OR = 3.23, 95% CI: 1.10-9.47, $P = 0.032$), while borderline results were considered negative, Mpox seropositivity remained significantly associated with a history of blood transfusion (OR = 5.79, 95% CI: 1.27-26.33, $P = 0.023$) and with sharing sharp objects (OR = 4.55, 95% CI: 1.12-18.48, $P = 0.034$).

Discussion

This study is the first to assess MPXV seroprevalence among sex workers in Chiang Mai providing crucial insights into undetected or unreported infections in this key population. We found an overall seroprevalence of 4.6% among 262 sex workers, indicating likely exposure to Mpox without confirmed clinical diagnoses. These findings align with recent local research in the same cohort, which reported high rates of newly diagnosed HIV, syphilis, hepatitis B, and hepatitis C during the easing of COVID-19 lockdowns [14]. Together, these results suggest that limited access to testing and specialist care during the pandemic may have contributed to unrecognized Mpox and other sexually transmitted infections (STIs) in this vulnerable group.

Many traditional serological assays rely on antigens derived from *Vaccinia virus* (VACV) or generic orthopoxvirus lysates, which limits their ability to distinguish Mpox-specific immune responses. This shortcoming can lead to uncertain or inflated estimates of Mpox seroprevalence. To address this critical issue, we developed and employed an in-house ELISA targeting the E8L antigen- a highly immunogenic surface-binding protein unique to MPXV [15]. In a comprehensive evaluation by Hunt et al. [16], E8L demonstrated superior diagnostic performance over other MPXV and VACV antigens, achieving 98% sensitivity and 93% specificity in differentiating Mpox-exposed individuals from unexposed controls. This finding establishes E8L as a reliable marker for Mpox-specific antibodies and validates its use in our assay for accurate serosurveillance.

Compared with other studies globally, the seroprevalence observed in our study is comparable to the 5.1% reported in South Florida among unvaccinated individuals without known Mpox infection [8], 7.4% among an MSM cohort in Berlin [7], and 7.4% among PLWH without prior vaccination or diagnosis in Rome [9]. However, our observed seroprevalence is lower than rates reported in some other studies that often utilize broadly reactive anti-orthopoxvirus assays. For example, an urban Brazilian study found a 16.9% prevalence of anti-orthopoxvirus neutralizing antibodies [17], and studies among MSM in the Netherlands reported VACV-specific antibody seroprevalence rates of 45.4% and 47.1% [18].

A notable finding from our study is the higher seroprevalence observed among males (5.2%) compared to females (4.0%), with an additional 3.4% showing borderline results exclusively among males (6.6%). This statistically significant difference ($P = 0.011$) indicates a gender disparity in Mpox exposure among sex worker in Chiang Mai, reflecting global epidemiological trends in which most Mpox cases have been reported among gay, bisexual, or MSM populations [18–21]. Supporting the observation, a serosurvey in South Florida found that seropositivity rates was 7.8% among males compared to 1.2% among females [8].

Mpox transmission in Thailand presents distinct challenges compared to high-income countries. Legal and social stigma surrounding sex work and LGBTQ+ identities can deter healthcare access and delay diagnosis, particularly among migrant sex workers facing barriers like legal status and lack of insurance. During the 2022 study period, Mpox diagnostics and antivirals such as tecovirimat were not publicly available, contributing to likely underreporting. Supporting this, MPXV DNA was also detected in wastewater in Bangkok prior to confirmed cases [10]. These findings stress the importance of community-based surveillance tailored to regional realities, where transmission pathways may extend beyond MSM-focused patterns seen elsewhere.

The observed higher seropositivity among male sex workers in our study aligns with global trends and regional evidence indicating elevated Mpox exposure within LGBTQ populations. In many Asian societies, including Thailand, stigma toward same-sex behavior, compounded by legal and cultural marginalization, can discourage healthcare engagement and limit the visibility of Mpox cases in official surveillance systems. A recent community-based study in Thailand using discarded condoms as a surveillance tool demonstrated high levels of undiagnosed Mpox and STI circulation among MSM populations, reinforcing the limitations of conventional facility-based reporting in such contexts [13].

We also analyzed the risk factors associated with Mpox IgG seropositivity among sex workers. Male gender emerged as a significant factor in the multivariable model when borderline results were considered positive, with an OR of 3.23. This aligns with global epidemiological patterns from the 2022 Mpox outbreak, which disproportionately affected MSM populations [18–21]. A systematic review and meta-analysis by Ugwu et al. [22] reported that identifying as MSM was significantly associated with Mpox transmission, (OR: 2.18) and Kupritz et al. [8] similarly found higher seropositivity among males (7.8% vs 1.2%) in a South Florida cohort. Together, these findings highlight a syndemic dynamic in which Mpox and other STI's are concentrated within overlapping male sexual networks [22].

The history of blood transfusion was strongly associated with Mpox seropositivity under both borderline interpretations: (OR = 4.40, borderline as positive) and (OR = 5.79, borderline as negative). Although Mpox is primarily transmitted through close physical or sexual contact, bloodborne transmission is not a recognized primary route. This association may instead reflect underlying vulnerabilities or overlapping risk behaviors. For instance, a history of blood transfusion could relate to past medical conditions or correlate with practices such as injection drug use, which are known to increase the risk of bloodborne infections like HIV and Hepatitis C. Previous study has detected Mpox DNA in the blood of infected individuals, indicating a theoretical risk of blood-borne transmission, even though no confirmed cases via transfusion have been documented to date [23].

Sharing sharp objects also emerged as a significant independent risk factor for Mpox seropositivity (OR = 4.55, borderline consid-

Table 2
Factors associated with Mpox IgG seropositivity among sex workers.

Characteristics		Mpox IgG positivity (borderline considered as positive)					Mpox IgG positivity (borderline considered as negative)				
		n/N (%)	Univariable		Multivariable		n/N (%)	Univariable		Multivariable	
			OR (95% CI)	*P*-value	OR (95% CI)	*P*-value		OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Mpox Ab positivity											
Gender	Female	5/126 (4.0)	1.00				5/126 (4.0)	1.00			
	Male	16/136 (11.8)	3.23 (1.15-9.09)	**0.027**	3.23 (1.10-9.47)	**0.032**	7/136/ (5.2)	1.31 (0.41-4.25)	0.649		
Age group (y)	<20	2/12 (16.7)	1.00				1/12 (8.3)	1.00			
	20-30	12/113 (10.6)	0.59 (0.12-3.04)	0.532			6/113 (5.3)	0.62 (0.07-5.60)	0.668		
	31- 40	4/96 (4.2)	0.22 (0.04-1.34)	**0.100**		N.S.	3/96 (3.1)	0.35 (0.03-3.71)	0.387		
	41-50	3/41 (7.3)	0.39 (0.06-2.69)	0.343			2/41 (4.9)	0.56 (0.05-6.82)	0.652		
Race	Thai	14/193 (7.3)	1.00				9/193 (4.7)	1.00			
	Non-Thai	7/69 (10.1)	1.44 (0.56-3.74)	0.450			3/69 (4.4)	0.93 (0.24-3.54)	0.914		
Education	Under College/University	20/238 (8.4)	1.00				11/238 (4.6)	1.00			
	College/University	1/24 (4.2)	0.47 (0.06-3.70)	0.476			1/24 (4.2)	0.90 (0.11-7.27)	0.919		
Workplace: Karaoke	No	20/225 (8.9)	1.00				12/225 (5.3)	1.00			
	Yes	1/37 (2.7)	0.28 (0.04-2.19)	**0.227**		N.S.	0/37 (0.0)	N/A			
Workplace: Pub bar, restaurants, rural bar, cafe	No	13/187 (7.0)	1.00				7/187 (3.7)	1.00			
	Yes	8/75 (10.7)	1.60 (0.63-4.03)	0.320			5/75 (6.7)	1.84 (0.56-5.98)	0.313		
Workplace: Massage parlor	No	20/218 (9.2)	1.00				12/218 (5.5)	1.00			
	Yes	1/44 (2.3)	0.23 (0.30-1.76)	**0.157**		N.S.	0/44 (0.0)	N/A			
Workplace: Traditional massage, spa sauna	No	16/190 (8.4)	1.00				9/190 (4.7)	1.00			
	Yes	5/72 (6.9)	0.81 (0.29-2.30)	0.695			3/72 (4.2)	0.87 (0.23-3.33)	0.844		
Stay in Thailand (y)	< 10	4/42 (9.5)	1.00				3/42 (7.1)	1.00			
	>10	17/220 (7.7)	0.80 (0.25-2.49)	0.695			9/220 (4.1)	0.55 (0.14-2.14)	0.392		
Marital status	Single	13/178 (7.3)	1.00				8/178 (4.5)	1.00			
	Having a partner	6/53 (11.3)	1.62 (0.58-4.49)	0.354			3/53 (5.7)	1.28 (0.33-4.99)	0.727		
	Separated/ Divorced/ Widowed	2/31 (6.5)	0.88 (0.19-4.08)	0.865			1/31 (3.2)	0.71 (0.09-5.87)	0.749		
Have children	No	16/136 (11.8)	1.00				8/136 (5.9)	1.00			
	Yes	5/126 (4.0)	0.31 (0.11-0.87)	**0.027**		N.S.	4/126 (3.2)	0.52 (0.15-1.79)	0.302		

(*continued on next page*)

Table 2 (*continued*)

Characteristics		Mpox IgG positivity (borderline considered as positive)					Mpox IgG positivity (borderline considered as negative)				
		n/N (%)	Univariable		Multivariable		n/N (%)	Univariable		Multivariable	
			OR (95% CI)	*P*-value	OR (95% CI)	*P*-value		OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Family members (persons)	1	6/106 (5.7)	1.00				5/106 (4.7)	1.00			
	2-3	9/88 (10.2)	1.90 (0.65-5.56)	**0.242**		N.S.	4/88 (4.6)	0.96 (0.25-3.70)	0.955		
	4-5	5/59 (8.5)	1.54 (0.45-5.29)	0.490			2/59 (3.4)	0.71 (0.13-3.77)	0.687		
	≥6	1/9 (11.1)	2.08 (0.22-19.49)	0.520			1/9 (11.1)	2.53 (0.26-24.31)	0.423		
Type of accommodation	House	7/70 (10.0)	1.00				5/70 (7.1)	1.00			
	Dormitory	1/46 (2.2)	0.20 (0.02-1.68)	**0.139**		N.S.	0/46 (0.0)	N/A			
	Rent a room	11/138 (8.0)	0.78 (0.29-2.11)	0.624			6/138 (4.4)	0.59 (0.17-2.01)	0.399		
	Other	2/8 (25.0)	3 (0.51-17.80)	**0.227**		N.S.	1/8 (12.5)	1.86 (0.19-18.23)	0.595		
Monthly income (THB)	≤15,000	14/150 (9.3)	1.00				9/150 (6.0)	1.00			
	>15,000	7/112 (6.3)	0.65 (0.25-1.66)	0.366			3/112 (2.7)	0.43 (0.11-1.63)	**0.215**		N.S.
Smoking	No	8/134 (6.0)	1.00				6/134 (4.5)	1.00			
	Yes	13/128 (10.2)	1.78 (0.71-4.45)	**0.217**		N.S.	6/128 (4.7)	1.05 (0.33-3.34)	0.935		
Alcohl consumption	No	5/39 (12.8)	1.00				3/39 (7.7)	1.00			
	Yes	16/223 (7.2)	0.53 (0.18-1.53)	**0.238**		N.S.	9/223 (4.0)	0.50 (0.13-1.95)	0.322		
Drug used	No	17/207 (8.2)	1.00				12/207 (5.8)	1.00			
	Yes	4/55 (7.3)	0.88 (0.28-2.72)	0.820			0/55 (0.0)	N/A			
Ever used drug injection	No	20/246 (8.1)	1.00				11/246 (4.5)	1.00			
	Yes	1/16 (6.3)	0.75 (0.09-6.00)	0.789			1/16 (6.3)	1.42 (0.17-11.78)	0.743		
Ever been diagnosed with STIs	No	10/135 (7.4)	1.00				6/135 (4.4)	1.00			
	Yes	7/108 (6.5)	0.87 (0.32-2.36)	0.779			3/108 (2.8)	0.61 (0.15-2.52)	0.498		
Ever had surgery	No	17/187 (9.1)	1.00				9/187 (4.8)	1.00			
	Yes	4/73 (5.5)	0.58 (0.19-1.78)	0.342			3/73 (4.1)	0.85 (0.22-3.22)	0.808		
	I don't know	0/2 (0.0)	N/A				0/2 (0.0)	N/A			
Ever had blood transfusion	No	15/236 (6.4)	1.00				8/236 (3.4)	1.00			
	Yes	5/22 (22.7)	4.33 (1.41-13.36)	**0.011**	4.40 (1.35-14.25)	**0.013**	4/22 (18.2)	6.33 (1.74-23.07)	**0.005**	5.79 (1.27-26.33)	**0.023**
	I don't know	1/4 (25.0)	4.91 (0.48-50.11)	**0.179**	3.49 (0.33-37.14)	0.299	0/4 (0.0)	NA			
Ever had needle stick	No	19/244 (7.8)	1.00				10/244 (4.1)	1.00			
	Yes	2/14 (14.3)	1.97 (0.41-9.47)	0.396			2/14 (14.3)	3.90 (0.77-19.81)	**0.101**		N.S.
	I don't know	0/4 (0.0)	N/A				0/4 (0.0)	N/A			

(*continued on next page*)

Table 2 (*continued*)

Characteristics		Mpox IgG positivity (borderline considered as positive)					Mpox IgG positivity (borderline considered as negative)				
		n/N (%)	Univariable		Multivariable		n/N (%)	Univariable		Multivariable	
			OR (95% CI)	*P*-value	OR (95% CI)	*P*-value		OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Ever had sharing sharp objects with others	No	9/155 (5.8)	1.00				3/155 (1.9)	1.00			
	Yes	12/107 (11.2)	2.05 (0.83-5.05)	**0.119**		N.S.	9/107 (8.4)	4.65 (1.23-17.61)	**0.024**	4.55 (1.12-18.48)	**0.034**
Piercing	No	6/68 (8.8)	1.00				3/68 (4.4)	1.00			
	Yes	15/194 (7.7)	0.87 (0.32-2.33)	0.776			9/194 (4.6)	1.05 (0.28-4.01)	0.938		
Tattoo	No	6/101 (5.9)	1.00				4/101 (4.0)	1.00			
	Yes	15/161 (9.3)	1.63 (0.61-4.34)	0.331			8/161 (5.0)	1.27 (0.37-4.32)	0.704		
Sexual orientation	Heterosexual	5/119 (4.2)	1.00				5/119 (4.2)	1.00			
	Homosexual	1/15 (6.7)	1.63 (0.18-14.96)	0.666			0/15 (0.0)	N/A			
	Bisexual	15/128 (11.7)	3.03 (1.06-8.61)	**0.038**		N.S.	7/128 (5.5)	1.32 (0.41-4.27)	0.644		
Age at first sexual intercourse (y)	>15	13/205 (6.3)	1.00				6/205 (2.9)	1.00			
	≤15	8/57 (14.0)	2.41 (0.95-6.14)	**0.065**		N.S.	6/57 (10.5)	3.90 (1.21-12.61)	**0.023**	2.78 (0.74-10.47)	0.131
Duration in sex work (y)	≤5	13/106 (12.3)	1.00				8/106 (7.6)	1.00			
	>5	8/156 (5.1)	0.39 (0.15-0.97)	**0.043**		N.S.	4/156 (2.6)	0.32 (0.09-1.01)	**0.071**	0.27 (0.07-1.03)	0.056
Vaginal sex	No	1/15 (6.7)	1.00				0/15 (0.0)	1.00			
	Yes	20/247 (8.1)	1.23 (0.15-9.87)	0.843			12/247 (4.9)	N/A			
Insertive anal sex	No	5/129 (3.9)	1.00				5/129 (3.9)	1.00			
	Yes	16/133 (12.0)	3.39 (1.20-9.55)	**0.021**		N.S.	7/133 (5.3)	1.38 (0.43-4.46)	0.593		
Receptive anal sex	No	17/211 (8.1)	1.00				11/211 (5.2)	1.00			
	Yes	4/50 (8.0)	0.99 (0.32-3.09)	0.989			1/50 (2.0)	0.37 (0.05-2.94)	0.348		
Number of sexual partner per day in the past 3 mo (persons)	0-1	18/177 (10.2)	1.00				12/177 (6.8)	1.00			
	>1	3/85 (3.5)	0.32 (0.09-1.13)	**0.077**		N.S.	0/85 (0.0)	N/A			
Sex with partner (husband or wife)	No	13/143 (9.1)	1.00				7/143 (4.9)	1.00			
	Yes	8/119 (6.7)	0.72 (0.29-1.80)	0.484			5/119 (4.2)	0.85 (0.26-2.76)	0.789		
Condom use with partner (husband or wife)	All the time	1/23 (4.4)	1.00				0/23 (0.0)	1.00			
	Occasionally or never	7/95 (7.4)	1.75 (0.20-14.97)	0.609			5/95 (5.3)	N/A			
Sex with client, in the past month	No	2/18 (11.1)	1.00				0/18 (0.0)	1.00			
	Yes	19/244 (7.8)	0.68 (0.14-3.16)	0.618			12/244 (4.9)	N/A			
Condom use with client, in the past month	All the time	15/184 (8.2)	1.00				8/184 (4.4)	1.00			
	Occasionally or never	4/60 (6.7)	0.80 (0.26-2.53)	**0.710**	0.58 (0.20-1.67)	0.312	4/60 (6.7)	1.57 (0.46-5.42)	**0.474**	0.61 (0.16-2.39)	0.481

Bold values represent $p < 0.25$, which were set as the selection criterion for inclusion in the multivariable analysis.

ered negative). Although Mpox is primarily transmitted through direct contact with lesions or bodily fluids, contaminated sharp objects may serve as a potential route of transmission by introducing infected material through the skin. Similar to blood transfusion, this behavior is a known risk factor for other bloodborne infections and may reflect broader vulnerabilities linked to high-risk practices. Supporting this, Waddell et al. [24] reported that individuals experiencing homelessness in San Francisco with suspected undiagnosed Mpox often share unwashed utensils and smoking devices, highlighting the syndemic context in which shared sociobehavioral risk facilitates the spread of multiple infections in marginalized populations.

The observed associations between Mpox seropositivity and both a history of blood transfusion and sharing sharp objects may reflect broader syndemic vulnerabilities, including the increasing prevalence of recreational and injecting drug use in Thailand. Although only a small proportion of participants reported injecting drug use, underreporting is likely to be due to stigma and legal consequences. Needle-sharing is well documented among Thai drug users and has been strongly linked to HIV and HCV transmission [25]. These behaviors, particularly needle-sharing, may theoretically serve as indirect pathways for MPXV transmission through contaminated instruments, especially when skin lesions are present. Moreover, the rising use of amphetamine-type stimulants (ATS) in urban Thai settings, often associated with high-risk sexual behavior, further compounds the risk of overlapping Mpox and STIs exposure [26].

Although our study did not find a statistically significant association between condom use and Mpox seropositivity, it is important to interpret this finding in the context of global transmission dynamics. The 2022 Mpox outbreak was predominantly driven by sexual contact, particularly among individuals with high-frequency sexual exposure. A recent seroprevalence study in Berlin identified that having more than two condomless anal sexual partners in the past 6 mo was significantly associated with Mpox seropositivity, emphasizing the role of unprotected sexual activity in transmission risk [7]. However, contrasting evidence from a study in northern Taiwan found that having multiple sexual partners was associated with lower odds of Mpox infection (aOR: 0.22) [21]. These differing findings suggest that the relationship between sexual behavior and Mpox transmission may be influenced by contextual factors such as population characteristics, local outbreak intensity, and awareness or engagement in protective behaviors. Therefore, while condom use alone may not offer complete protection due to Mpox's ability to spread via skin-to-skin and lesion contact, comprehensive prevention strategies should still emphasize safer sexual practices, symptom recognition, and early diagnosis.

During the 2022 outbreak, Thailand primarily reported cases caused by Clade IIb, which is associated with more efficient human-to-human transmission, particularly via sexual contact and close physical interaction. Clade IIb infections often present with mucocutaneous lesions in the oropharyngeal or anorectal regions, areas that may not be protected by condom use during sexual activity. This biological characteristic may explain why our study did not find a statistically significant association between condom use and Mpox IgG seropositivity. Recent global wastewater surveillance studies also point to Clade IIb's dominant role in sexual transmission, especially in low-resource and conflict-affected regions, reinforcing the need to consider clade-specific pathogenesis in designing prevention strategies [12].

In addition, the early phase of the 2022 Mpox outbreak in Thailand was marked by the lack of access to specific antiviral treatments such as Tecovirimat, which has been the primary therapeutic agent recommended for severe or high-risk cases. As Tecovirimat was not widely accessible during our study period (March-December 2022), undiagnosed or mild cases would have remained untreated and unrecorded, emphasizing the critical need for equitable access to both diagnostics and therapeutics in future outbreak responses.

Our findings, supported by existing literature, highlight the multifactorial nature of Mpox transmission, extending beyond sexual contact to include broader behavioral and social determinants. These insights underscore the need for targeted interventions for sex workers, including enhanced surveillance, comprehensive risk communication, and integrated services addressing Mpox, HIV, and other STIs.

This study has some limitations

First, the data were derived from a pre-existing dataset originally collected for STIs surveillance, not specifically designed for Mpox seroprevalence assessment. As a result, important variables such as Mpox infection history and vaccination status were unavailable, limiting the contextual interpretation of seropositivity. Second, similar to the approach used by Hongjaisee et al. [14], behavioral data were obtained through self-reported face-to-face interviews, which may be subject to recall and social desirability bias. Finally, given the cross-sectional nature of seroprevalence data, the findings reflect cumulative past exposure and cannot determine the temporality of risk behaviors or pinpoint recent transmission dynamics.

Consequently, anti-E8L antibodies may not definitively differentiate natural MPXV infection from immune responses elicited by historical smallpox vaccination. However, given that Thailand ceased routine smallpox immunization in the late 1970s, and the median age of participants in our cohort was 31 y (IQR: 25-38), the likelihood of prior smallpox vaccination among study subjects is negligible. Thus, the detected IgG seropositivity likely reflects MPXV exposure rather than cross-reactive immunity from smallpox vaccination.

Taken together, our findings must be interpreted in the context of potential alternative sources of orthopoxvirus antibody positivity. While the E8L antigen used in our ELISA is highly immunogenic and MPXV-specific, it cannot fully distinguish between antibodies resulting from MPXV infection and those derived from prior exposure to Vaccinia-based vaccines. However, smallpox vaccination was discontinued in Thailand by the late 1970s, and none of the study participants fall within the likely vaccinated age range. Additionally, MVA-based vaccines were not officially available in Thailand or neighboring countries during the study period, and no evidence suggests informal access. Furthermore, although individual-level data on Mpox diagnoses or vaccination was unavailable, Thailand reported sporadic Mpox cases during late 2022, suggesting possible undetected transmission in this population. These epidemiological and contextual factors support the interpretation that the detected seropositivity reflects true MPXV exposure rather than vaccination-induced cross-reactivity.

Conclusion

In conclusion, our study presents novel Mpox-specific seroprevalence data among sex workers in Chiang Mai, Thailand, using the E8L antigen to enhance diagnostic specificity. The observed 4.6% seroprevalence offers a more accurate estimate of Mpox exposure in this key population, in contrast to the potential overestimation seen with non-specific orthopoxvirus assays. These findings underscore the utility of targeted serological assays in accurately assessing the true burden of Mpox and informing tailored public health interventions.

Author contributions

Conceptualization: Sayamon Hongjaisee, Nicole Ngo-Giang-Huong, Woottichai Khamduang; Methodology: Muhammad Umer, Kriangkrai Chawansuntati, Jiraprapa Wipasa, Kanyaruck Jindaphun, Kamonporn Kotemul, Yee Mon Thant; Investigation: Sayamon Hongjaisee, Kriangkrai Chawansuntati, Jiraprapa Wipasa, Nicole Ngo-Giang-Huong, Woottichai Khamduang; Supervision: Sayamon Hongjaisee, Kriangkrai Chawansuntati, Woottichai Khamduang; Data curation: Muhammad Umer, Sayamon Hongjaisee, Kriangkrai Chawansuntati, Woottichai Khamduang; Formal analysis: Muhammad Umer, Sayamon Hongjaisee, Kriangkrai Chawansuntati, Woottichai Khamduang; Resources: Sayamon Hongjaisee; Project administration: Sayamon Hongjaisee; Validation: Muhammad Umer, Sayamon Hongjaisee, Kriangkrai Chawansuntati, Jiraprapa Wipasa, Nicole Ngo-Giang-Huong, Woottichai Khamduang; Visualization: Muhammad Umer; Writing—original draft: Muhammad Umer, Sayamon Hongjaisee; Writing—review & editing: Muhammad Umer, Sayamon Hongjaisee, Woottichai Khamduang, Nicole Ngo-Giang-Huong, Kriangkrai Chawansuntati, Jiraprapa Wipasa, Kanyaruck Jindaphun, Kamonporn Kotemul, Yee Mon Thant.

Declaration of competing interest

The authors declare no conflict of interest.

Funding

This study was supported by the Research Institute for Health Sciences, Chiang Mai University (Grant No. 029/2024), and the student research fund under the CMU Presidential Scholarship, Chiang Mai University, Thailand (Grant No. 8393(25) /3457).

Ethical statement

This study was approved by the Human Experimentation Committee, Research Institute for Health Sciences, Chiang Mai University (Certificate of Ethical Approval No. 55/2024).

Acknowledgments

The authors would like to acknowledge the School of Health Sciences Research, Research Institute for Health Sciences, Chiang Mai University, for providing the student research fund. We also thank the Research Center for Molecular and Cell Biology, Research Institute for Health Sciences, and LUCENT International Collaboration, Faculty of Associated Medical Sciences, Chiang Mai University, along with their team members, for facilitating the research work.

References

[1] Nuzzo JB, Borio LL, Gostin LO. The WHO declaration of monkeypox as a global public health emergency. *Jama* 2022;**328**(7):615–17.
[2] Haque MA, Halder AS, Hossain MJ, Islam MR. Prediction of potential public health risk of the recent multicountry monkeypox outbreak: an update after the end declaration of global public health emergency. *Health Sci. Rep.* 2024;**7**(6):e2136.
[3] Thornhill J, Gandhi M, Orkin C. Mpox: the reemergence of an old disease and inequities. *Ann. Rev. Med.* 2024;**75**(1):159–75.
[4] Singh P, Sridhar SB, Shareef J, Talath S, Mohapatra P, Khatib MN, et al. The resurgence of monkeypox: epidemiology, clinical features, and public health implications in the post-smallpox eradication era. *N. Microbes N. Infect.* 2024:101487.
[5] Martínez JI, Montalbán EG, Bueno SJ, Martínez FM, Juliá AN, Díaz JS, et al. Monkeypox outbreak predominantly affecting men who have sex with men. *Eurosurveillance* 2022;**27**(27):2200471.
[6] Rodriguez-Diaz CE, Crowley JS, Santiago-Rivera Y, Millett GA. From COVID-19 to monkeypox: unlearned lessons for black, Latino, and other men with HIV who have sex with men. *Am. J. Public Health* 2022;**112**(11):1567–71.
[7] Marcus U, Michel J, Lunchenkov N, Beslic D, Treindl F, Surtees R, et al. A seroprevalence study indicates a high proportion of clinically undiagnosed MPXV infections in men who have sex with men in Berlin, Germany. *BMC Infect. Dis.* 2024;**24**(1):1153.
[8] Kupritz J, Pahwa S, Pallikkuth S. Serosurvey of immunity to monkeypox (mpox) virus antigens in people living with HIV in South Florida. *Pathogens* 2023;**12**(11):1355.
[9] Salvo PF, Sanfilippo A, Baldin G, Iannone V, Ciccullo A, Farinacci D, et al. Investigating seroprevalence of IgG antibodies against Monkeypox Virus (MPXV) in a cohort of people living with HIV (PLWH) in Rome, during the 2022 outbreak: moving beyond traditional at-risk populations. *Travel Med. Infect. Dis.* 2024;**62**:102757.
[10] Wannigama DL, Amarasiri M, Hongsing P, Hurst C, Modchang C, Chadsuthi S, et al. Multiple traces of monkeypox detected in non-sewered wastewater with sparse sampling from a densely populated metropolitan area in Asia. *Sci. Total Environ.* 2023;**858**:159816.
[11] Wannigama DL, Amarasiri M, Phattharapornjaroen P, Hurst C, Modchang C, Chadsuthi S, et al. Tracing the transmission of mpox through wastewater surveillance in Southeast Asia. *J. Travel Med.* 2023;**30**(5):taad096.
[12] Wannigama DL, Amarasiri M, Phattharapornjaroen P, Hurst C, Modchang C, Wang Y, et al. Global participatory wastewater surveillance to understand mpox clade diversity in war and conflict-affected countries. *J. Travel Med.* 2025;**32**(5):taaf052.
[13] Wannigama DL, Amarasiri M, Phattharapornjaroen P, Hurst C, Modchang C, Besa JJV, et al. Community-based mpox and sexually transmitted disease surveillance using discarded condoms in the global south. *Lancet Infect. Dis.* 2024;**24**(10):e610-e613.
[14] Hongjaisee S, Khamduang W, Kham-Kjing N, Ngo-Giang-Huong N, Tangmunkongvorakul A. Seroprevalence and associated factors of HIV, syphilis, hepatitis B, and hepatitis C infections among sex workers in Chiang-mai, Thailand during easing of COVID-19 lockdown measures. *PLoS One* 2024;**19**(12):e0316668.
[15] Shantier SW, Mustafa MI, Abdelmoneim AH, Fadl HA, Elbager SG, Makhawi AM. Novel multi epitope-based vaccine against monkeypox virus: vaccinomic approach. *Sci. Rep.* 2022;**12**(1):15983.
[16] Hunt JH, Jones JL, Gebo KA, Hansoti B, Traut CC, Hamill MM, et al. Discordant performance of mpox serological assays. *J. Virologic. Methods* 2024;**329**:115004.
[17] de Oliveira JS, Barbosa Costa G, Stoffella Dutra AG, Domingos IJDS, Costa PSPMd, Silva PHBe, et al. Low prevalence of anti-orthopoxvirus neutralizing antibodies in an urban population of Brazil. *J. Med. Virol.* 2023;**95**(6):e28859.
[18] Shamier MC, Zaeck LM, Götz HM, Vieyra B, Verstrepen BE, Wijnans K, et al. Scenarios of future mpox outbreaks among men who have sex with men: a modelling study based on cross-sectional seroprevalence data from the Netherlands, 2022. *Eurosurveillance* 2024;**29**(17):2300532.
[19] Minhaj FS, Singh V, Cohen SE, Townsend MB, Scott H, Szumowski J, et al. Prevalence of undiagnosed monkeypox virus infections during global mpox outbreak. *Emerg. Infect. Dis.* 2023;**29**(11):2307.
[20] Fifer H, Otter A, Yung M, Pitt R, Everall I, Jones S, Rampling T, Pereira S, McEwan A, Mohammed H, Brown C, Harris R, Sinka K, Houlihan C, Saunders J. P031 Prevalence of Mpox-specific antibodies in sera collected between January – September 2022 in England. A cross-sectional seroprevalence study. *Sexually Trans Infec* 2023;**99**(Suppl 1):A38–A38. doi:10.1136/sextrans-BASHH-2023.71.
[21] Chen W-Y, Cheng S-H, Chen C-P, Lee S-Y, Ku S-Y, Gan S-T, et al. Risk factors for Mpox infection among MSM with STIs in Northern Taiwan, 2022–2024. *J. Infect. Chemother.* 2025:102750.
[22] Ugwu CLJ, Bragazzi NL, Wu J, Kong JD, Asgary A, Orbinski J, et al. Risk factors of human Mpox (Monkeypox) infection: a systematic review and meta-analysis. *medRxiv* 2024:2024.08.
[23] Dodd RY, Stramer SL. Monkeypox and transfusion safety. *Transfus. Med. Rev.* 2023;**37**(1):7–9.
[24] Waddell CJ, Filardo TD, Prasad N, et al. Possible Undetected Mpox Infection Among Persons Accessing Homeless Services and Staying in Encampments — San Francisco, California, October–November 2022. *MMWR Morb Mortal Wkly Rep* 2023;**72**:227–31. doi:10.15585/mmwr.mm7209a3.
[25] Perngmark P, Celentano DD, Kawichai S. Needle sharing among southern Thai drug injectors. *Addiction* 2003;**98**(8):1153–61.
[26] Sutcliffe CG, Aramrattana A, Sherman SG, Sirirojn B, German D, Wongworapat K, et al. Incidence of HIV and sexually transmitted infections and risk factors for acquisition among young methamphetamine users in northern Thailand. *Sex. Trans. Dis.* 2009;**36**(5):284–9.