PO4/01

A SELF-SAMPLING SCREENING STRATEGY ADDRESSED TO ASYMPTOMATIC OR UNRECOGNIZED MONKEYPOX VIRUS INFECTION IN GAY, BISEXUAL, AND OTHER MEN WHO HAVE SEX WITH MEN AND TRANS WOMEN IN SPAIN

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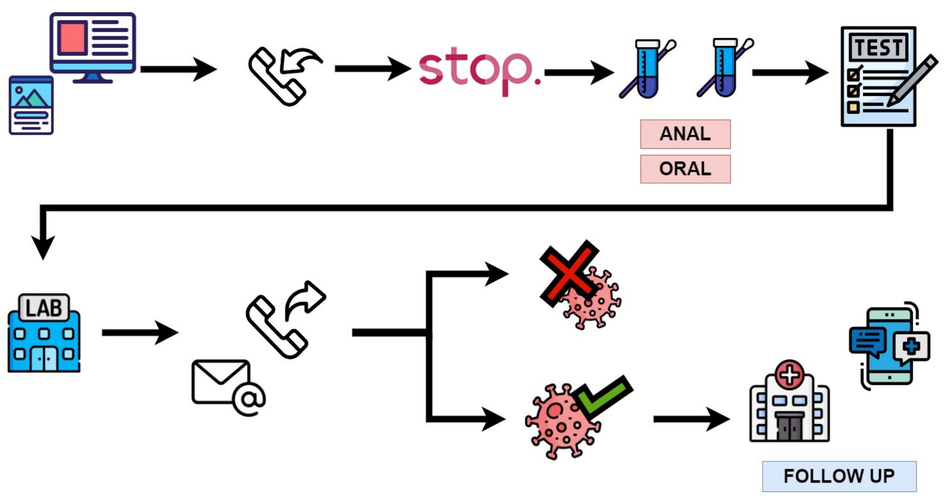
Introduction:
Mpox is a zoonotic disease caused by monkeypox virus (MPXV), a virus belonging to the Orthopoxvirus genus, which is endemic in several African countries[1]. From 1 January through 12 December 2022, a cumulative total of 82,628 laboratory-confirmed cases of Mpox and 65 deaths were reported to the World Health Organization (WHO) from 110 countries[2]. On 23 July 2022 the WHO declared Mpox to be a Public Health Emergency of International Concern3. Spain, with 7,412 cases, has been the third-most affected country after the United States of America and Brazi[l2]. In Spain, as in other countries, the outbreak has mainly affected gay, bisexual and other men who have sex with men (GBMSM) with no documented history of travel to countries where MPXV is endemic.

Objectives:
In the present study, we aimed (i) to assess the prevalence of MPXV infection among highly exposed GBMSM and trans women (TW), asymptomatic or with mild unrecognized Mpox symptoms, who were recruited in a community-based centre in Barcelona, (ii) to assess the presence of replication-competent particles of MPXV and (iii) to evaluate the feasibility and acceptability of a community-based self-sampling strategy for Mpox diagnosis.



Methods:
We implemented a transversal non-randomized study offering free self-sampling kits for MPXV testing through a collaborating community centre that offers voluntary counselling and testing for HIV (STOP, Barcelona, Spain). The field coordinator communicated test results to participants by a phone call. The study targeted: GBMSM and TW. Inclusion criteria were: Self-identification as GBMSM or TW, over 18 years old, with no symptoms of MPXV infection and considered at high risk of contracting Mpox. High risk was defined as: GBMSM and TW who are sex workers and/or chemsex users and/or who practice group sex and/or are HIV positive or are PrEP users. The self-sampling kits included an anal and a pharyngeal swab), pre-labelled swab containers and a brochure with detailed instructions with pictures explaining how to get the samples. A video with the instructions of sample collection was available on YouTube and was accessible. After obtaining the sample, the swabs were immediately placed in 1 ml of transport medium and samples were stored at 4ºC. A parcel courier service provided the secondary and tertiary containers, and samples were transported at 4ºC to the reference laboratory (Microbiology Department, Clinical Laboratory Nord Metropolitan Area, Germans Trias i Pujol University Hospital). We analyzed all samples for the detection of MPXV DNA with a real-time PCR-based assay (qualitative and quantitative) at the reference laboratory. Anal and pharyngeal swabs were tested by MPXV real-time PCR and positive samples were inoculated into Vero E6 cells, which were subsequently checked for MPXV infection by cytopathic effect (CPE) and anti-vaccinia pAb staining by FACS and confocal microscopy. All identifying data collected was encrypted.

Results:
From August to October 2022, 113 individuals were recruited at a community centre in Barcelona and participated in the study. From all the participants, 89 (78.76%) were cis men, 17 (15.04%) were TW and 3 (2.65%) non-binary gender. The median age of participants was 35.0 years (Interquartile Range (IQR): 30.0-43.0), 96 (85.02%) individuals were gay or bisexual and 72 (63.72%) were migrants. Additionally, 44 (38.94%) participants self-reported HIV infection and among HIV negative participants 41 (59.42%) were on PreP and 58 (51.33%) had had an STI in the previous 12 months. Regarding MPXV, 28 (24.78%) participants had had contact with a confirmed Mpox case over the previous 30 days. Also seven (6.19%) and 13 (11.50%) participants had received the smallpox vaccine in their childhood or in the previous 12 months, respectively. The median number of sexual partners of participants over the previous 30 days was 5.00 (IQR: 1.00-10.00), of the total participants 42 (39.25%) had not used condoms during sexual intercourse over the previous month and 38 (33.63%) had had sex in exchange for money, gifts or favours. Furthermore, 29 (30.85%) had practiced chemsex in the previous 30 days and three (5.45%) had practiced slamming in the last month. Eight positive MPXV results for seven individuals were detected and we estimated a total prevalence of 6.19% (95% CI: 1.75%-10.64%). All positive participants were cis gay men and prevalence in this group was 7.87% (95% CI: 2.27%-13.46%).The characteristics of the participants with a positive MPXV result are shown in Table 3. Five participants tested positive in pharyngeal swabs, one in the anal swab and one in the pharyngeal and the anal swabs. Three out of the six PCR positive samples successfully cultured over time (40, 66 and 95) were positive, not only for CPE at the indicated day of harvesting, but also for staining for specific anti-vaccinia pAb detected by FACS and confocal microscopy. We also tested the recovered viral stocks by PCR, which yielded positive results for MPXV, and we sequenced them along with the positive control viral stock, which confirmed the specificity of the MPXV presence in cell cultures, while we detected no signal in the negative control.

Table 3. Results of MPXV infection among participants Stop Mpox. August - October 2022. Barcelona (Spain)

		PCR Results			Cell Culture							
Individual	Location	Result	Ct	CV (Copies/mL)	Culture viability	Day of Harvesting	CPE	IF	FACS	Ct	$TCID_{50}$/ml	Symptoms
2	Pharingeal	Positive	34.9	18300	No	4	N/A	N/A	N/A	N/A	N/A	
	Anal	Negative	-	-	N/A	N/A	N/A	N/A	N/A	N/A	N/A	No reported symptoms
40	Pharingeal	Positive	30.1	347000	Yes	8	Positive	Positive	8.41%	21.96	10^4,3	No symptoms before testing.
	Anal	Negative	-	-	N/A	N/A	N/A	N/A	N/A	N/A	N/A	After testing the participant reported: Fever, exhaustation, sore throat and a skin lesion
64	Pharingeal	Positive	37.09	4827	Yes	8	Negative	Negative	Negative	Negative	Negative	
	Anal	Negative	-	-	N/A	N/A	N/A	N/A	N/A	N/A	N/A	No available information
66	Pharingeal	Positive	24.85	8532000	Yes	7	Positive	Positive	74.2%*	20.42	10^4,8	
	Anal	Positive	35.35	13960	No	2	N/A	N/A	N/A	N/A	N/A	Before testing: A swallen inguinal lymph node
72	Pharingeal	Negative	-	-	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Before testing: Fever, exhaustation and
	Anal	Positive	38.06	2674	Yes	7	Positive	Positive	3.75%	35.98	<10^1	a skin lesion in the genital area
81	Pharingeal	Positive	36.99	5126	Yes	8	Negative	Negative	Negative	Negative	Negative	
	Anal	Negative	-	-	N/A	N/A	N/A	N/A	N/A	N/A	N/A	No reported symptoms
95	Pharingeal	Positive	36.79	5825	Yes	14	Positive	Positive	3.18%	25.88	10^2,8	
	Anal	Negative	-	-	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Skin lesions in the genital area
112	Pharingeal	Negative	-	-	Yes	8	Negative	Negative	Negative	Negative	Negative	-
113	Pharingeal	Negative	-	-	Yes	8	Negative	Negative	Negative	Negative	Negative	-
Control +	Viral stock	N/A	-	-	Yes	7	Positive	Positive	Positive	19.21	10^6,8	-
Control -	Culture media	N/A	-	-	Yes	8	Negative	Negative	Negative	Negative	Negative	-

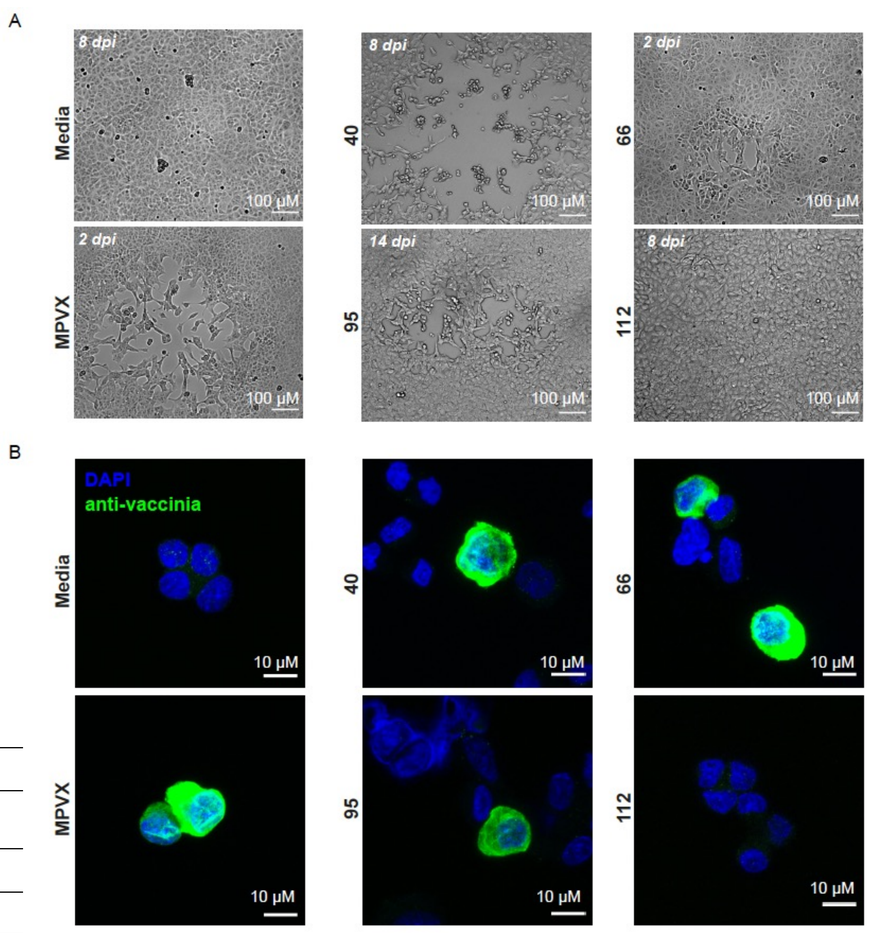


Figure 1: Representative results from samples assayed to isolate infectious MPVX. A. Optical microscopy images of Vero E6 cultures inoculated with swab samples. Images were taken at the day post infection (dpi) indicated in the top left part of the images. Scale bars correspond to 100 µm. Viral isolation was done only once due to sample availability B. Confocal microscopy images of cells where swab samples were grown after intracellular staining with the anti-vaccinia pAb revealed with an Alexa 488 secondary antibody from the cultures. DAPI staining is shown in blue (nuclei) and α-Vaccinia is shown in green. Scale bars correspond to 20 µm. Viral isolation was done only once due to sample availability

Conclusions:
Our findings have important public health implications, particularly for MPXV infection prevention and control policies. We have shown that MPXV infection is present among asymptomatic individuals and among vulnerable populations. We also confirmed that MPXV symptoms can overlap and be confused with other diseases, such as STIs. Moreover, we were able to isolate replication-competent viruses from pharyngeal and anal swabs from asymptomatic or mildly symptomatic patients.

Recommendations:
Educational interventions are needed to familiarize the members of vulnerable populations with the nature of MPXV symptoms and eradicate the associated stigma in order to increase awareness and health care seeking behaviour in these populations. In an epidemic scenario, early diagnosis by means of screening strategies should be aimed not only at suspected clinical cases and direct contacts, but also at all GBMSM at high risk of contracting Mpox, regardless of their symptoms. Community-based self-sampling tools can be acceptable and effective to increase early diagnosis and the eventual isolation of infectious cases. On the other hand, health care workers in STI clinics, primary care, and emergency rooms in other health care settings should be aware of the variety of Mpox symptoms and the possibility of asymptomatic cases before excluding Mpox as a potential diagnosis. Finally, stigma and discrimination in the most affected group, GBMSM, should be addressed to warrant equitable access to diagnosis, treatments and vaccines. More data are needed to better establish the attributable risk of asymptomatic infections in the transmission of MPXV in an outbreak, including seminal transmission.

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References: 1.Petersen, E., Kantele, A., Koopmans, M., Asogun, D. & Yinka-Ogunleye, A. Human Monkeypox Epidemiologic and Clinical Characteristics, Diagnosis, and Prevention. doi:10.1016/j.idc.2019.03.001 . 2. World Health Organization (WHO). *Multi-country outbreak of monkeypox*. *World Health Organization* (2022).

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IGTP Institut de Recerca Germans Trias i Pujol
Salut/ ICO Institut Català d'Oncologia
ciber ESP