DOI: 10.1002/jmv.29371

LETTER TO THE EDITOR

JOURNAL OF MEDICAL VIROLOGY WILEY

Mpox, herpes, and enteroviruses: Differential diagnosis

To the editor,

Mpox (formerly monkeypox) disease has been reported in Central and West Africa for years until May 2022 when the United Kingdom reported several cases with no evidence of epidemiological links to travel to endemic areas, nor any imports of animals or contact with other travellers' cases.[1] Since then, 116 countries have confirmed 91 788 cases.[2] Spain, after United States and Brazil, is the third country in number of cases (7647 at October 31, 2023).[2]

The National Center of Microbiology (NCM) as the National Reference Laboratory for zoonoses in Spain, carried out the diagnosis of 1196 suspected MPX cases until April 5, 2023, confirming the presence of Mpox virus (MPXV) by polymerase chain reaction (PCR) in 768 patients (64.2%) (unpublished data). The priority during the outbreak was to confirm the presence of MPXV to implement rapid treatment and isolation of the patient to control the spread of the disease. However, since MPXV is clinically indistinguishable from other pox-like illnesses, particularly smallpox and chickenpox, laboratory diagnosis is important. Moreover, varicella-zoster virus (VZV) has been reported coinfecting Mpox patients.[3–5]

Nucleic acid extracts available (342) from samples of patients with vesicular skin lesions suspected to be produced by Mpox were screened for herpes simplex virus (HSV), VZV, and enteroviruses (EVs) by real-time (RT) multiplex RT-PCR (Table 1). Of these, 252 were from swab samples of patients (199 men, 49 women, and four individuals with no gender data) with a previous negative result for Mpox (group 1) and 90 were from skin lesions (53), saliva (19), semen (7), serum (6), blood (2), urine (1), rectal (1), and pharyngeal (1) swabs in patients (all men) with a previous positive result for Mpox (group 2).

In group 1, 41 were positive for VZV (16.2%), 17 for HSV (6.7%), and four for EV (1.6%). For EV genotyping, specific RT-nested PCRs (amplifying and sequencing a 400 nt fragment of the VP1 region) for EV species A, B, and C were used, followed by sequencing and BLAST analysis.[6] Coxsakievirus A6 was identified in the samples of three patients and B2 in one patient. HSV was found in nine samples of the second group, being detected in saliva ($n = 6$), cutaneous lesions ($n = 2$), and semen ($n = 1$). Distribution of cases according to age is shown in Figure 1 (in group 2 all were in the range 30–39).

VZV was detected in more than 16% of the negative cases, which accounted for at least 3% of all patients with suspected Mpox infection, received at NCM. It was found in all age groups except for patients <10 years (although only eight patients in this group were analyzed). This virus had also been found in other studies, in both sporadic cases and series when differential diagnosis for suspected MPX cases was carried out, representing between 25% and 39% of the cases.[7–9] HSV and EV cases were also previously reported in suspected cases of MPXV.[9–11] Regarding EV infections, coxsackievirus A6 was identified in most of the EV infections, which is consistent with the fact that this EV is one of the more frequently associated with mucocutaneous diseases

TABLE 1 Primers and probes used for multiplex real-time polymerase chain reaction and amplification conditions.

Primer name	Sequence (5′–3′)
VZV	Forward: ATCGATCCATCAGCGGTCC
	Reverse: CCCCGCAAGACGTTTGG
	Probe: *VIC*-CGATCCGAGGATTCGTA-*MGB*
EV	Forward: ACAIGGTGYGAAGAGYCTATTGAGC
	Reverse: TGCTCCRIRGTTRGGATTAGC
	Probe: Texas red-CCTCCGGCCCCTGAATGCG-BHQ2
HSV1	Forward: GCGGTAGGCACAAAATTCGG
	Reverse: CCCCCATTGGGCTGTTG
HSV2	Forward: AGCGGTATGCGCAAAATTCG
	Reverse: CCCATCGGGCTGCTGG
	Probe for HSV: *FAM*-CGACAGTCGATAATC-*MGB*
Internal control	Forward: CAGATTAGCAATTGGTGCGAA
	Reverse: GTGGGCAAATCCGAGGAA
	Probe: *IRD-700*-AATGATTGGGCCACGTCACG-BHQ3
Cycling conditions	50°C/20 min
	Cycling 1: 6× touchdown −0.5°C annealing *T*: 94°C/20 s, 61–58°C/20 s, 72°C/20 s,
	Cycling 2: 40× (94°C/30 s, 58°C/90 s)

Abbreviations: EV, enterovirus; HSV, herpesvirus simplex; VZV, varicella-zoster virus.

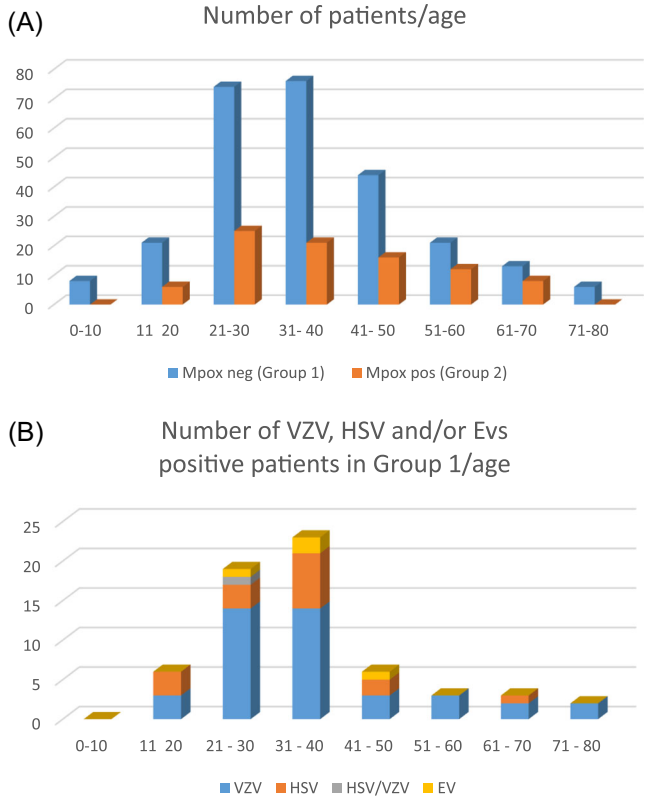

FIGURE 1 Age of the patients of the study. (A) Total number of cases studied by groups of age. (B) Positive cases for EV, HSV, and VZV by groups of age shown in colors. EV, enterovirus; HSV, herpesvirus simplex; VZV, varicella-zoster virus.

worldwide, being responsible for hand, foot, and mouth disease (HFMD) and unspecific exanthema.[12] Reports of HFMD-causing agents have been reported in Germany, Bolivia, and Argentina in the context of this outbreak.[10,11]

We also detected coinfection by Mpox and HSV in six saliva samples, two cutaneous lesions, and one sample of semen (Figure 1), in agreement with previously reported cases.[13] Coinfection between MPXV and VZV has been reported by several authors, some of which suggest that MPXV infection could be promoted by the alteration in the host immune response caused by VZV or that MPXV enters through VZV lesions.[4,5] A reasonable explanation of coinfection with HSV may be reactivation of latent HSV, although primary infection could not be ruled out.

According to our results, one of four cases suspected of having Mpox due to suggestive skin lesions is caused by other viruses that may share similar clinical presentations. Therefore, it is crucial to establish a definitive molecular diagnosis to ensure correct public health measures. These measures also include isolating the patient, vaccinating contacts to contain the outbreak, and administering antiviral antiherpetic treatment if necessary.

Broader studies including good clinical descriptions and a wider range of pathogens including some nonviral infections will help in the characterization of similar outbreaks if necessary.

AUTHOR CONTRIBUTIONS

María Paz Sánchez-Seco, Anabel Negredo, and David Tarragó designed the study. María Paz Sánchez-Seco and David Tarragó coordinated the study. Laura Guillén-Calvo, Patricia Sánchez-Mora, Paqui Molero, and María Cabrerizo performed the laboratory screening. Ana Vázquez, María Cabrerizo, Juan Ledesma, and David Tarragó analyzed sequencing data. Eva Orviz and Vicente Estrada provided most of the samples and attended the patients. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We would like to thank the technical work of Vanessa Recio and Laura Herrero. We are also very grateful to Monserrat Torres for her help in this outbreak. This research was supported by CIBER (Strategic Action for Monkeypox)—Consorcio Centro de Investigación Biomédica en Red—(CB 2021), Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación and Unión Europea—NextGenerationEU, and Centro de investigación en Red en Enfermedades infecciosas.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. The data presented in the manuscript have not been made available. They will be available when published.

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