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The 2024 Mpox surveillance in Senegal uncovers a large circulation of Chickenpox

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During preparedness activities in Senegal to the 2024 Mpox Public Health Emergency of International Concern, a study was conducted to assess the prevalence of Varicella-Zoster virus among patients suspected of having Mpox. Samples, including skin swabs, serum, and nasopharyngeal swabs, were collected from 103 patients who presented with Mpox-like symptoms. Molecular testing via *q*PCR revealed that 30.1% of patients tested positive for herpesviruses, whereas no Mpox cases were detected. Common symptoms include fever, skin rash, headache, and myalgia, which closely resemble Mpox symptoms, increasing the risk of misdiagnosis. The most affected group was children under 15 years of age (50% of herpesvirus cases), followed by adults over 30 years of age (30.8%). The male/female sex ratio among herpesvirus-positive patients was 2.1, indicating a higher prevalence in males. Phylogenetic analysis of 14 newly characterized Varicella-Zoster virus genomes from metagenomic sequencing revealed that the strains circulating in Senegal were closely related to those from Guinea-Bissau, suggesting possible regional transmission. In addition, viral and bacterial coinfections were identified in Mpox-negative patients, which may have contributed to some skin lesions initially suspected to be Mpox. Our data highlight the importance of differential diagnostic testing to distinguish between Mpox and other infections, such as Chickenpox. The unexpectedly high prevalence of herpesviruses among suspected Mpox cases underscores the need for improved laboratory diagnostics, enhanced epidemiological surveillance, and targeted public health interventions to prevent misdiagnosis and improve patient management.

Keywords Mpox, Varicella-Zoster virus, Herpesvirus, *q*PCR, Sequencing, Senegal

Mpox is a viral zoonotic disease sustained in humans through direct person-to-person transmission and previously endemic only to Africa[1]. The increasing incidence of this disease and its public health implications have attracted increased attention, particularly in the rainforest regions of Central and West Africa[2,3]. Mpox is caused by the monkeypox virus, a member of the genus *Orthopoxvirus*, the same genus that includes the variola virus responsible for smallpox[4–6]. The monkeypox virus has two main clades: clade Ⅰ and clade Ⅱ. Clade Ⅰ has subclades Ⅰa and Ⅰb, and clade Ⅱ has subclades Ⅱa and Ⅱb, where subclade Ⅱb is known to have caused a global outbreak from 2022 to 2023, whereas subclades Ⅰa and Ⅰb still pose health risks in 2024[7]. The disease manifests with symptoms similar to those of smallpox, including fever, rash, and lymphadenopathy, but generally has lower mortality rates[4]. The first documented case of human Mpox was reported in 1970 in the Democratic Republic of Congo (DRC), shortly after the global eradication of smallpox was declared[8]. This initial case marked the beginning of what would become a recurrent public health challenge in Africa. Over the

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past few decades, Mpox has caused numerous outbreaks, predominantly in countries such as the DRC, Nigeria, Cameroon, and the Central African Republic[9–11].

On August 14, 2024, the World Health Organization (WHO) declared the outbreak a Public Health Emergency of International Concern (PHEIC). This is the WHO's highest global alert level, and the decision recognizes the potential threat this virus poses to countries around the world[12]. Over 20 000 Mpox cases have been reported from 13 African Union Member States thus far in 2024, including 3 311 confirmed cases and 582 deaths (case fatality; CF 2.9%)[12]. The highest prevalence was recorded in the DRC, where monkeypox virus subclades Ia and Ib circulate, representing more than 90% of the cases reported on the African continent to date, with 19 667 cases (16 706 suspected and 2 961 confirmed), including 575 deaths (CF 2.9%) reported from all provinces[13]. Monkeypox virus clade I and clade II circulate in different countries on the continent[13]. As of 2024, confirmed Mpox cases due to subclades Ia or Ib have also been reported in five of the eight countries neighboring the DRC: the Republic of the Congo (21 confirmed cases and 141 suspected cases), the Central African Republic (45 confirmed cases), Burundi (190 confirmed cases and 512 suspected cases), Rwanda (four confirmed cases), and Uganda (three confirmed cases). In addition, Kenya reported one person infected with monkeypox virus clade Ib in 2024 and another where the clade is still unknown, and Gabon reported one person with Mpox on 22 August with travel history to Uganda[14].

The WHO African Regional Office (AFRO) reviewed and expanded the regional incident management support team to ensure that Member States receive the necessary support for managing the Mpox outbreak. A critical meeting held in South Africa discussed urgent measures to address the increasing number of Mpox cases[12]. During preparedness activities for the 2024 Mpox PHEIC, a national surveillance program focusing primarily on the Mpox was established by the Ministry of Health and Social Actions (MoHSA) in Senegal, where the disease is not endemic. Laboratory tests include differential diagnoses for other pathogens, such as herpesviruses, measles and rubella, which can cause similar clinical signs to improve patient management.

The herpesvirus family includes more than 100 types of viruses, eight which are well known to be infectious in humans. Among the eight viruses, the most common are herpes simplex virus type 1 (HSV1) and 2 (HSV2) and varicella-zoster virus (VZV)[15]. Globally, 64% of people under 50 years of age have HSV1, while 13% of people aged between 15 and 49 years have HSV2[16]. However, VZV is responsible for varicella or chickenpox disease, a neglected viral infection that was responsible for 83,963,744 infections worldwide in 2019[17]. Chickenpox is a highly contagious disease that mainly spreads from unvaccinated people with chickenpox to others who have never had the virus. Up to 90% of people who are not immune and close to someone with chickenpox will also become infected[18]. In children, it presents as a rash with fever and is a mild disease. However, in infants, adolescents, adults, elderly individuals, pregnant women, and immunocompromised individuals, the risk for more severe disease and a higher incidence of complications is high. After primary infection with varicella, the virus can remain dormant in the body during a latent phase and may reactivate later in life as herpes zoster (shingles), particularly as cell-mediated immunity declines with age[19]. In West Africa, data suggest that varicella (chickenpox) outbreaks may be sustained by multiple circulating viral genotypes rather than a single source, reflecting complex local transmission dynamics. A well-characterized outbreak in Guinea-Bissau revealed co-circulation of four distinct genotypes over a seven-month period, indicating continuous transmission even in tropical climates where seasonality is often considered less pronounced. Although this study did not explicitly analyze seasonal trends, it demonstrates that varicella can persist endemically in tropical African settings[20].

In this study, we reported not only the large circulation of VZV in 9 out of the 14 administrative regions in Senegal during the 2024 Mpox PHEIC but also the identification and genomic characterization of the genogroup A5 VZV from Mpox-negative patients via quantitative polymerase chain reaction (*q*PCR) and metagenomics to assess the implications of this circulation in public health.

Results

Descriptive characteristics of the included patients

From August to December 2024, a total of 103 patients were enrolled, and samples were screened for panels of viral etiologies via *q*PCR. Among these, 79 reported their age, with a median of 18 years (Q1 = 8.5 years, Q3 = 33.5 years), and ages ranged from 1 to 67 years. Children under 15 years of age accounted for 44.3% of the cases, followed by young adults (15–30, 24.1%) and adults over 30 years of age (31.6%). The male/female sex ratio was 1.75 (65/37) (missing data for 1 patient). Overall, no confirmed Mpox-positive cases were detected during the study period. However, the identified etiologies included herpesviruses and paramyxoviruses. Among the 103 patients tested, herpesviruses were detected in 30.1% (31/103), while measles virus and rubella virus were each detected in 2.9% (3/103) of patients (Table 1).

Spatial distribution of the identified etiologies

The geographical distribution shows a notable concentration of cases in 9 of the 14 regions in Senegal, including Dakar (Center, West, North, Guediawaye, Pikine, Keur Massar, Yeumbeul, Mbao), Saint-Louis (Richard-Toll, Pete, Matam), Diourbel (Touba), Louga (Sakal, Dahra, Linguere), Thies (Pout), Kaolack (Guinguineo), Tambacounda, Ziguinchor and Kedougou (Salemata). Herpesvirus cases were detected in a wider range of regions compared with measles and rubella, which were each identified in only three cases and limited to the Dakar, Thies, and Louga regions. The regions of Dakar (center), Diourbel (Touba), Saint-Louis (Richard-Toll, Matam) and Kaolack (Guinguineo) have a high prevalence of herpesvirus cases (Fig. 1). The regions with the highest herpesvirus prevalence, particularly Dakar and Touba, are characterized by exceptionally high population density Dakar reaches approximately 12,233 inhabitants/km^2, which facilitates person-to-person transmission through close contact[21]. Touba is the second most populated city in Senegal, with over one million inhabitants in 2023, and serves as a major religious and social hub, especially during the Magal, which brings together millions of attendees[22]. In contrast, regions such as Kaolack and Saint-Louis, while urban, maintain strong rural urban

	Overall ($n = 103$)
Age (years)	
N	79
N-Miss	24
Age group (years)	
<= 15	35 (44.3%)
[15–30]	19 (24.1%)
> 30	25 (31.6%)
Sex	
N-Miss	1
Male	65 (63.7%)
Female	37 (36.3%)
Mpox	
Positive	0
Negative	103 (100.0%)
Measles	
Negative	100 (97.1%)
Positive	3 (2.9%)
Rubella	
Negative	100 (97.1%)
Positive	3 (2.9%)
Herpesvirus	
Negative	72 (69.9%)
Positive	31 (30.1%)

Table 1. Descriptive characteristics of the patients included in this study. **N**: Number, **N-miss**: Number of missing values.

connectivity, potentially enabling the spread of infections to surrounding rural areas *via* transport and trade networks[23].

Clinical signs in herpesvirus-positive patients

The main symptoms observed in herpesvirus-positive patients included skin rash, fever, headache, myalgia, asthenia, dysphagia, chills, cough, and sweating. However, no vomiting, pruritus, arthralgia or lymphadenopathy was found in herpesvirus-positive patients. Analysis of percentages or frequencies revealed that skin rash and fever are the most common symptoms in both herpesvirus-negative and herpesvirus-positive patients (Fig. 2).

Temporal distribution of herpesvirus-positive cases

The temporal distribution of suspected Mpox cases and confirmed herpesvirus-positive cases between August and December 2024 showed minimal daily counts on most days (< 5). A short plateau was observed in late August, followed by a brief rise in early September before returning to a low background circulation. This pattern does not indicate a sustained increasing trend but rather a transient fluctuation in case numbers (Fig. 3).

Descriptive characteristics of herpesvirus-positive patients

A total of 31 patients out of the included patients tested positive for herpesvirus DNA by *q*PCR. The male/female sex ratio was 21/10 (2.1). Children under 15 years of age accounted for 50% of the cases, followed by young adults (15 to 30, 19.2%) and adults over 30 years of age (30.8%). The age distribution was not significantly different between herpesvirus-positive and herpesvirus-negative patients ($p = 0.714$). In males, there was a slightly greater proportion of herpesvirus-positive patients (67.7%) than herpesvirus-negative patients (62.0%), although this difference was not statistically significant ($p = 0.658$). The results for measles and rubella coinfections remained similar in both groups. Furthermore, no significant association was found between herpesvirus cases and positivity for measles or rubella (Table 2).

The herpesviruses detected among positive cases included VZV, EBV, HHV7, HSV1, HHV6, and CMV. Among the 31 herpesvirus-positive patients, 21 (60.0%) had VZV, 4 (11.4%) had EBV, 4 (11.4%) had HHV7, 3 (8.6%) had HSV1, 2 (5.7%) had HHV6, and 1 (2.9%) had CMV (Fig. 4). A total of four herpesvirus coinfection was observed in our cohort: EBV with HHV6, HHV7 with HSV1, VZV with EBV, and VZV with HSV1.

The herpesvirus-positive patients were treated using oral acyclovir (with 200 mg five times per day) for an average period of 10 days, combined with topical antiseptic care.

Genomic data

Of the 31 herpesvirus-positive patients identified by *q*PCR, 28 had Ct values < 29 and were selected for sequencing. Among these, 12 tested positives for VZV DNA. In addition, VZV DNA was detected in two patients who had

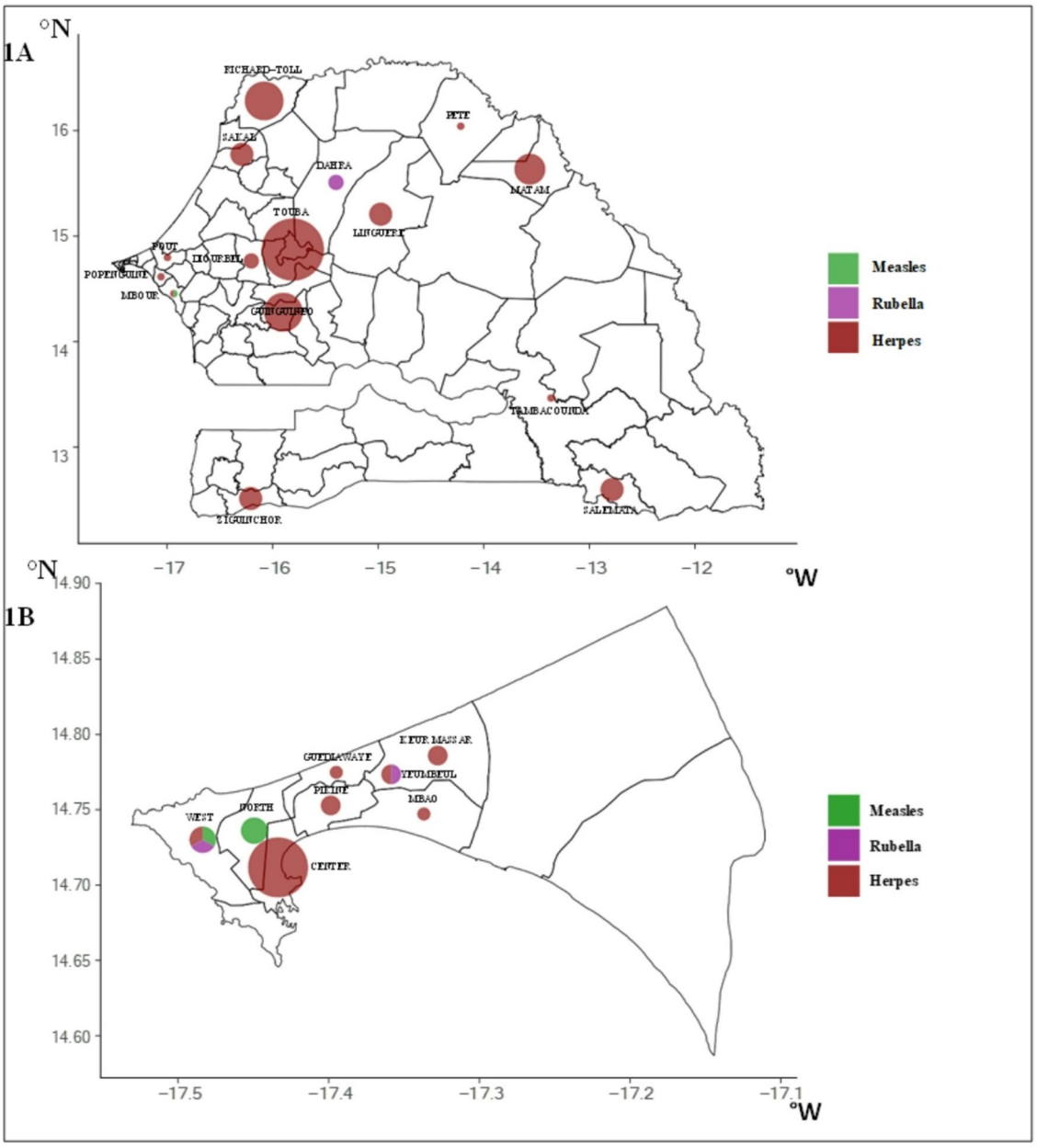

Fig. 1. Spatial distribution of identified etiologies. (**A**) shows the national-level spatial distribution of detected cases by pathogen; (**B**) provides an enlarged view of the Dakar region to illustrate finer-scale distribution.

initially tested negative for herpesvirus by *q*PCR. While herpesvirus cases were confirmed, no Mpox cases were identified through sequencing.

Ten complete *Brucella anthropi* sequences, four complete *Staphylococcus aureus* sequences, four complete *Staphylococcus pyogenes* sequences, three complete *Staphylococcus epidermidis* sequences, one complete *Staphylococcus hominis* sequence, one complete *Pseudomonas putida* sequence and one complete *Brucella* sp. sequence were successively generated via Nextera metagenomic sequencing of the *q*PCR-negative samples. A sequence of *Molluscum contagiosum* virus (MOCV) subtype 2 closes to MH320556.1 (by **nblast**: https://blast.ncbi.nlm.nih.gov/Blast.cgi) was also identified in one of the *q*PCR-negative skin swab samples (Table 3).

Phylogenetic analysis

In this study, a total of thirteen near-complete (81–90% coverage) and one partial (27% coverage) VZV sequence were successively generated via enrichment-based and metagenomics sequencing methods from *q*PCR-positive and *q*PCR-negative samples (skin swabs, nasopharyngeal swabs, and serum), respectively. The generated VZV sequences were submitted to NCBI GenBank under the accession numbers PV211163 to PV211175 (Supplementary Table A). The ML phylogenetic tree was inferred via IQtree online software with the best nucleotide substitution model for our dataset (Fig. 5). The newly characterized VZV genomes from Senegal

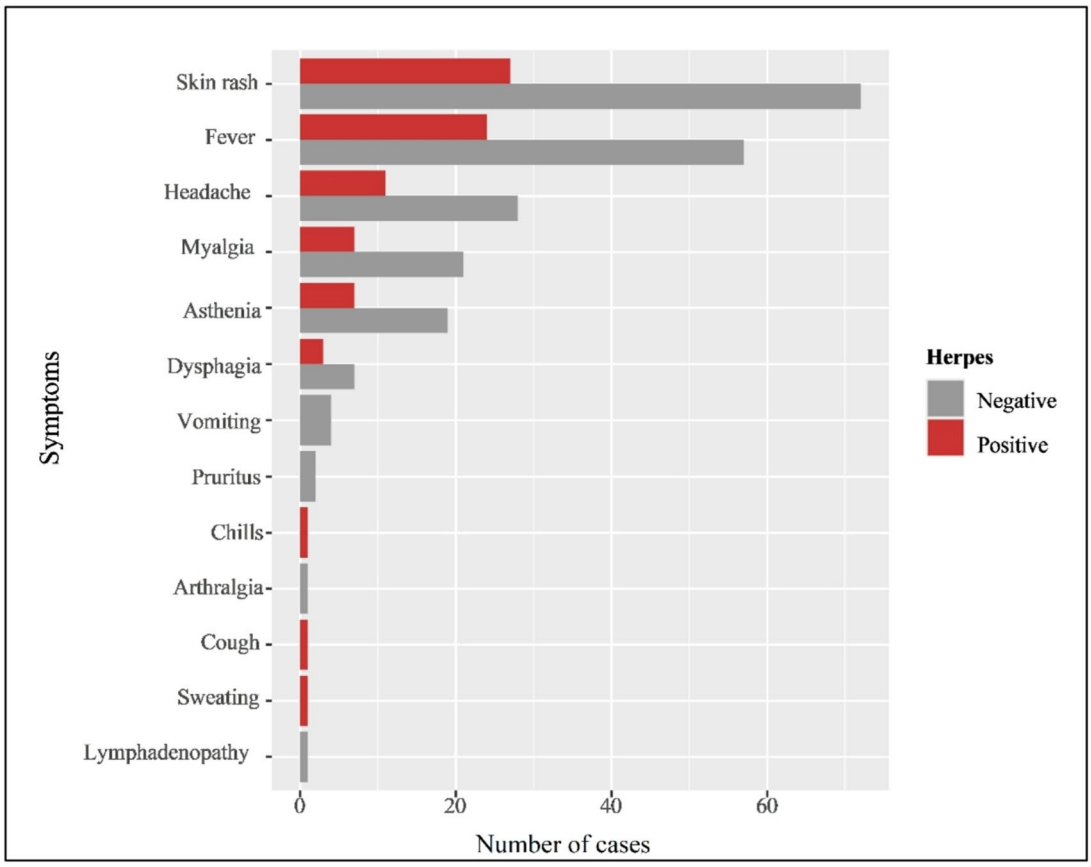

Fig. 2. Clinical symptoms reported among the 103 patients included in the study, stratified by herpesvirus status (positive vs. negative).

were aligned with VZV sequences previously available in GenBank. Most of the genomes obtained grouped with the VZV genome from Guinea-Bissau (**KM355717.1**), which belongs to clade 5 (genogroup 5 A) and was first identified from an 18-year-old patient in 2001, during a large chickenpox outbreak in Guinea-Bissau[20].

Discussion

Mpox is a zoonotic viral disease caused by the monkeypox virus, a member of the *Orthopoxvirus* genus. During August 2024, a significant increase in Mpox-suspected cases and confirmed cases of VZV was observed. This trend was similarly noted in Burundi, where a notable number of VZV cases were reported, mainly among children aged 1 to17 years[24]. Generally, the temporal distribution of VZV in Senegal varies, with epidemic peaks potentially occurring in March and coinciding with measles and rubella surveillance efforts in Senegal[25].

We also observed variation in the geographical distribution of Mpox-suspected cases in Senegal, with the majority located in the Dakar region, which is the most populated city, as noted in countries that declared Mpox outbreaks in 2024, such as Burundi and DRC[26,27]. These regional patterns of outbreaks highlight the importance of targeted surveillance and health interventions in high-risk areas.

In Senegal, the median age of herpesvirus-positive patients was 15 years, with children under 15 years of age accounting for 50% of the cases. The sex distribution also revealed a male (67.7%) predominance of herpesvirus cases compared with females (32.3%). VZV was the most represented herpesvirus, accounting for 66.7% of male cases and 70.0% of female cases among herpesvirus-positive patients. Similarly, in DRC, males constitute a slight majority, and the median age of VZV-positive patients is 18.14 years, indicating a predominance among younger populations[28]. At the onset of the 2022 Mpox outbreak, screening for any suspected case was recommended: children were historically the age group most affected, were most at risk of developing a severe form, and were at risk of rapid spread through direct contact and droplets at school[29]. The clinical distinction in cases of atypical rash between cases linked to Mpox and cases linked to chickenpox was difficult[29]. In Nigeria, during the 2017–2020 epidemic, the predominance of Mpox in favor of males was justified by the fact that most households in Nigeria are highly patriarchal, where males are generally breadwinners and risk-takers and are therefore more exposed to infected animal and human hosts[30].

Our data revealed that 30.1% of the 103 included patients had herpesvirus infections, with a higher prevalence of VZV (60%). These data resonate with previous findings from Brazil, where differential diagnostic testing revealed that 22.36% of Mpox-negative samples tested positive for VZV[31]. In both contexts, the high prevalence of VZV highlights the importance of comprehensive diagnostic testing to avoid misclassification of

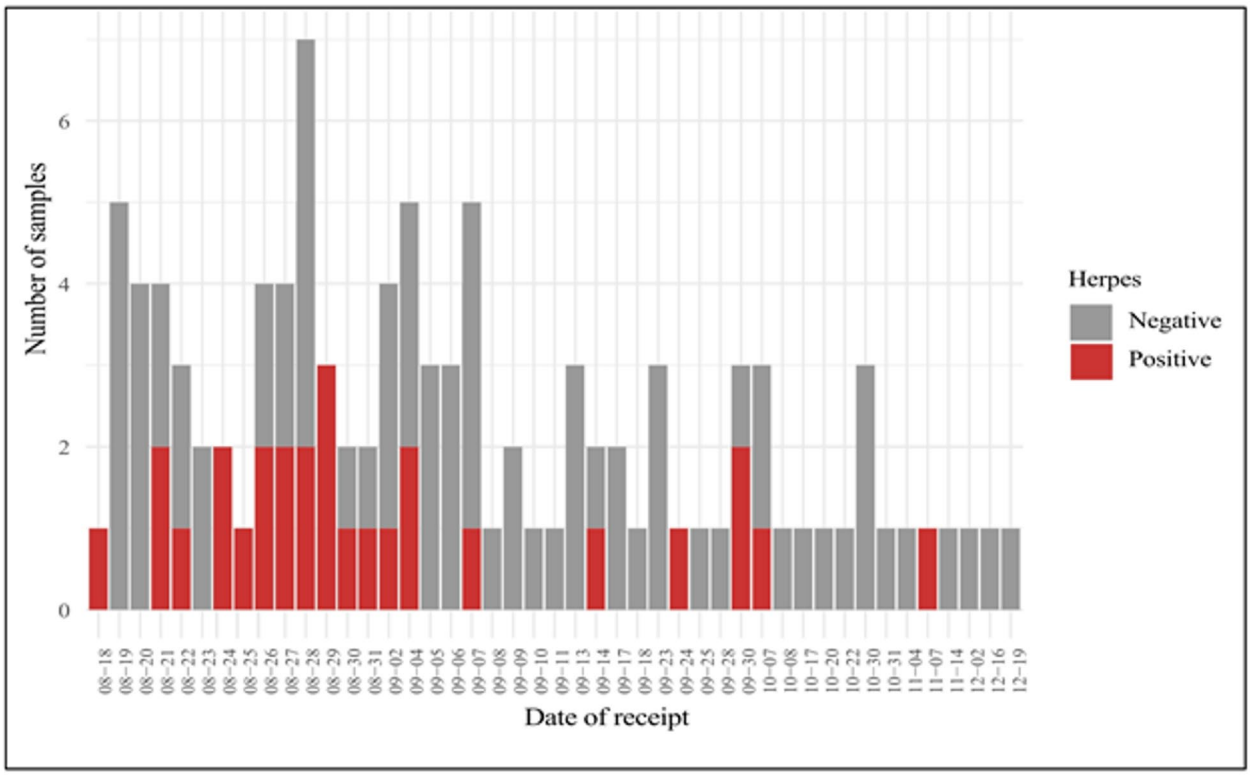

Fig. 3. Temporal distribution of suspected Mpox cases ($n = 103$) and herpesvirus-positive cases ($n = 31$) recorded between August and December 2024.

	Negative ($n = 72$)	Positive ($n = 31$)	Total ($n = 103$)	*p* value
Age (years)				0.210
N-Miss	19	5	24	
Median	18.0	15.0	18.0	
Q1, Q3	9.0, 35.0	6.2, 31.7	8.5, 33.5	
Min	2.0	1.0	1.0	
Max	67.0	48.0	67.0	
Age group (years)				0.714
N-Miss	19	5	24	
<= 15	22 (41.5%)	13 (50.0%)	35 (44.3%)	
[15–30]	14 (26.4%)	5 (19.2%)	19 (24.1%)	
> 30	17 (32.1%)	8 (30.8%)	25 (31.6%)	
Sex				0.658
N-Miss	1	0	1	
Male	44 (62.0%)	21 (67.7%)	65 (63.7%)	
Female	27 (38.0%)	10 (32.3%)	37 (36.3%)	
Measles				1.000
Positive	2 (2.8%)	1 (3.2%)	3 (2.9%)	
Negative	70 (97.2%)	30 (96.8%)	100 (97.1%)	
Rubella				0.552
Positive	3 (4.2%)	0 (0.0%)	3 (2.9%)	
Negative	69 (95.8%)	31 (100.0%)	100 (97.1%)	

Table 2. Sociodemographic and clinical characteristics of all patients included in the study ($n = 103$), presented by herpesvirus status (positive vs. negative). **N**: Number, **N-miss**: Number of missing values.

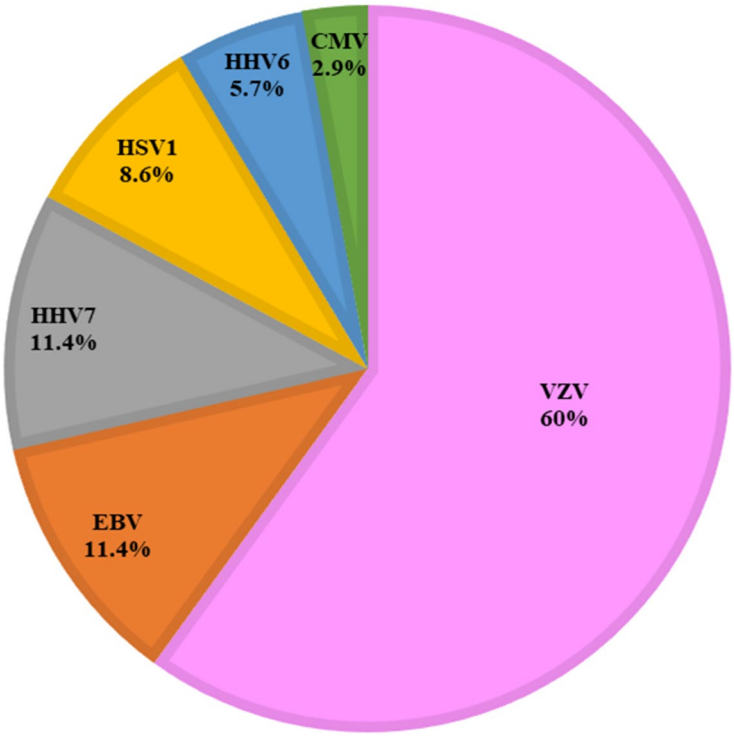

Fig. 4. Distribution of herpesvirus types. The colors indicate the proportions of each type of herpesvirus detected.

Mpox. In addition, symptoms such as rash and fever are common among VZV-positive individuals, emphasizing the diagnostic overlap with Mpox. VZV is a highly contagious virus that enters the body through the respiratory tract and can spread via fomites from varicella and shingle skin lesions[32]. Since Mpox and VZV infections are anatomically similar in their macule and papule presentations in their early stages[33], this could complicate their diagnosis and patient management. In addition to VZV, other herpesviruses, including EBV (11.4%), HHV7 (11.4%), HSV1 (8.6%), HHV6 (5.7%), and CMV (2.9%), have been detected. This has been observed in most differential tests conducted during Mpox outbreaks. In a retrospective study of samples tested for Mpox *via* a syndromic molecular assay for vesicular viruses, the most frequently detected virus was HSV2 (11%), followed by VZV (5.5%), HSV1 and HHV6 (2.8%)[34]. These findings highlight the importance of differential diagnosis in suspected Mpox cases. Thus, there is a crucial need to train clinicians in the course of both diseases. Although measles cases have been increasing in Senegal since early 2024, affecting 41% of the country, only a few suspected Mpox cases in this study were confirmed as measles or rubella. This may reflect the fact that clinicians are generally well trained to recognize the classic signs of measles and rubella, which allows them to rule out these diseases more readily during the differential diagnosis of Mpox-like rashes. However, measles and rubella can present with confluent maculopapular rash and Forchheimer's spots, as previously observed during the 2022 Mpox PHEIC[35].

In addition to VZV, we also identified subtype 2 MOCV from a patient, a virus causing similar signs and symptoms in humans, highlighting the importance of differential diagnosis[36]. MOCV is a viral cutaneous infection that occurs worldwide in childhood. Since the elimination of smallpox, poxvirus has continued to circulate exclusively in humans[37]. This identification highlights the importance of metagenomics in the surveillance of diseases to increase the detection spectrum, which should be promoted in future public health studies. The use of a metagenomics method tailored for detecting pathogens in infectious diseases allowed us to generate thirteen near-complete and one partial VZV sequence from skin swabs, nasopharyngeal swabs, and serum samples. Phylogenetic analysis via ML revealed that these fourteen newly characterized VZV sequences from Senegal clustered with a strain previously identified in March 2001 during a large chickenpox outbreak from an 18-year-old patient living in the Bandim peri-urban area (KM355717.1) in Bissau Guinea, which borders Senegal, suggesting possible virus import[20]. This suggests a possible regional transmission pattern, highlighting the interconnected nature of viral spread within West Africa. These findings underscore the necessity of cross-border surveillance and genomic epidemiology for understanding viral evolution and controlling future outbreaks. The VZV sequence from Bissau Guinea belongs to clade 5 and genogroup 5 A. Although the clade 5 lineage was previously reported in Africa and Southeast Asia[20], the full-genome sequences characterized in our study represent, to date, the first from Senegal and could be useful not only in future phylodynamic studies but also in the development of diagnostic or medical countermeasures.

Bacterial superinfections have been observed in VZV-negative patients, which may also be responsible for the skin lesions that may be mistaken for Mpox, leading to potential misdiagnoses. Conditions such as ecthyma,

Pathogen detected	*q*PCR-negative-samples (*n* = 57)	*q*PCR-positive-herpesvirus samples (*n* = 28)	Total (*n* = 85)	*p*-value
Varicella-zoster virus				1.335e-05
Positive	2 (3.5%)	12 (42.9%)	14 (16.5%)	
Negative	55 (96.5%)	16 (57.1%)	71 (83.5%)	
Cytomegalovirus				0.329
Positive	0 (0.0%)	1 (3.6%)	1 (1.2%)	
Negative	57 (100.0%)	27 (96.4%)	84 (98.8%)	
Herpes simplex virus 1				0.033
Positive	0 (0.0%)	3 (10.7%)	3 (3.5%)	
Negative	57 (100.0%)	25 (89.3%)	82 (96.5%)	
Epstein–Barr virus				0.010
Positive	0 (0.0%)	4 (14.3%)	4 (4.7%)	
Negative	57 (100.0%)	24 (85.7%)	81 (95.3%)	
Human herpesvirus 6				0.105
Positive	0 (0.0%)	2 (7.1%)	2 (2.4%)	
Negative	57 (100.0%)	26 (92.9%)	83 (97.6%)	
Human herpesvirus 7				0.010
Positive	0 (0.0%)	4 (14.3%)	4 (4.7%)	
Negative	57 (100.0%)	24 (85.7%)	81 (95.3%)	
***Molluscum contagiosum*virus**				1.000
Positive	1 (1.8%)	0 (0.0%)	1 (1.2%)	
Negative	56 (98.2%)	28 (100.0%)	84 (98.8%)	
Brucella anthropi				0.026
Positive	10 (17.5%)	0 (0.0%)	10 (11.8%)	
Negative	47 (82.5%)	28 (100.0%)	75 (88.2%)	
Staphylococcus aureus				0.297
Positive	4 (7.0%)	0 (0.0%)	4 (4.7%)	
Negative	53 (93.0%)	28 (100.0%)	81 (95.3%)	
Staphylococcus pyogenes				0.297
Positive	4 (7.0%)	0 (0.0%)	4 (4.7%)	
Negative	53 (93.0%)	28 (100.0%)	81 (95.3%)	
Staphylococcus epidermidis				0.547
Positive	3 (5.3%)	0 (0.0%)	3 (3.5%)	
Negative	54 (94.7%)	28 (100.0%)	82 (96.5%)	
Pseudomonas putida				1.000
Positive	1 (1.8%)	0 (0.0%)	1 (1.2%)	
Negative	56 (98.2%)	28 (100.0%)	84 (98.8%)	
Staphylococcus hominis				1.000
Positive	1 (1.8%)	0 (0.0%)	1 (1.2%)	
Negative	56 (98.2%)	28 (100.0%)	84 (98.8%)	
***Brucella sp*JSBl001**				1.000
Positive	1 (1.8%)	0 (0.0%)	1 (1.2%)	
Negative	56 (98.2%)	28 (100.0%)	84 (98.8%)	

Table 3. Pathogens identified by metagenomic sequencing in *q*PCR-negative and *q*PCR-positive samples. **n**: Number. Note: "Negative" and "Positive" refer to detection of the listed pathogen by metagenomic sequencing.

caused by pathogens such as *Staphylococcus aureus* and group A beta-hemolytic *Streptococcus*, present ulcerative plaques that can closely resemble Mpox lesions. A case series highlighted instances where ecthyma was initially suspected to be Mpox due to similar clinical presentations[38]. During the 2022 Mpox PHEIC, previous data indicated that the prevalence of bacterial superinfections among hospitalized patients ranged from 3% to 11%[39]. Given the potential severity of these superinfections, it is crucial to monitor and treat them promptly in patients with Mpox to prevent serious complications. This approach aligns with the WHO's recommendations, which emphasize supportive care, including the prevention and management of secondary infections, as a cornerstone of Mpox treatment[40]. Thus, vigilant monitoring and timely management of bacterial superinfections are essential components of Mpox care to mitigate the risk of severe complications.

Our study highlights the importance of differential diagnostic testing to distinguish Mpox from other infections with skin rashes, such as herpesviruses. The unexpectedly high prevalence of VZV found in Mpox-suspected cases in our study emphasizes the need for enhanced laboratory diagnostics, epidemiological

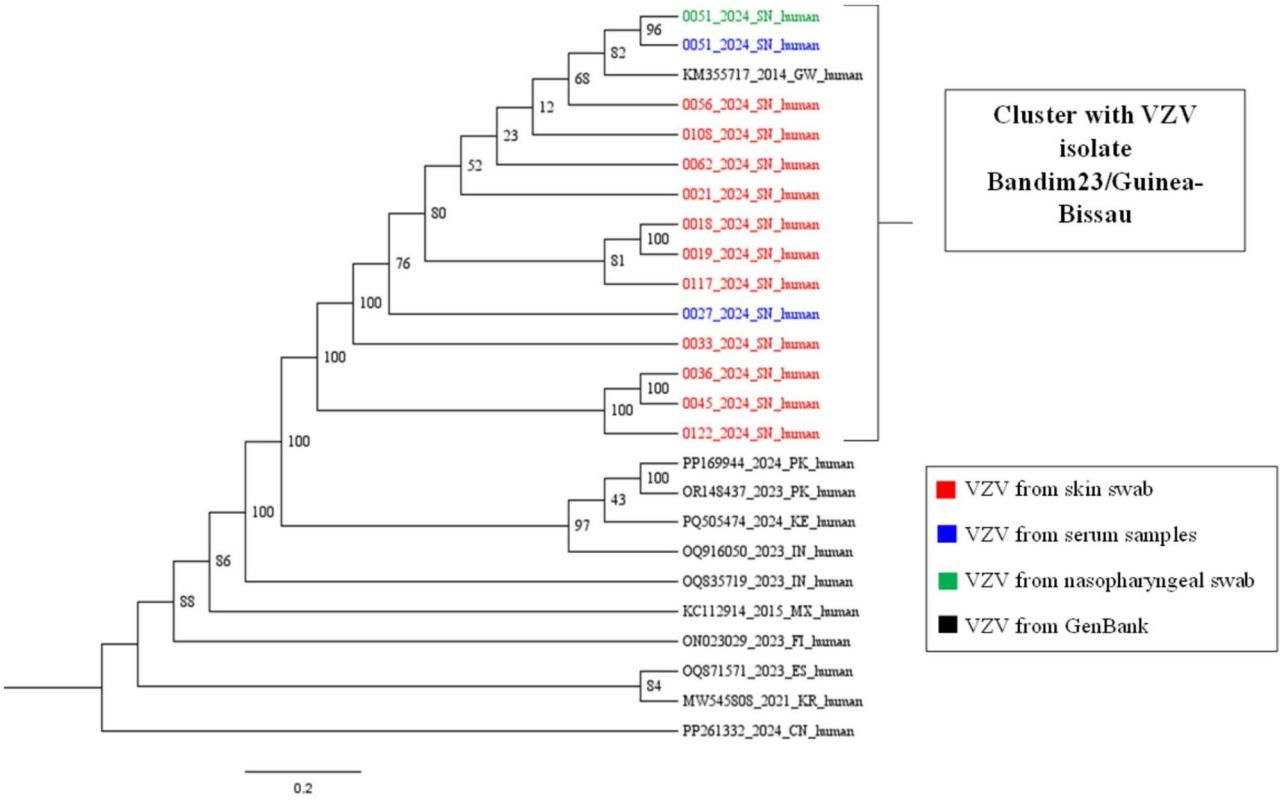

Fig. 5. Maximum likelihood tree based on the partial VZV sequence 0108_2024_SN_human (118 310 nt). The nodes are labeled with bootstrap values, and the names of the VZV isolates are color coded as follows: in **red**, VZV isolated from skin swab samples from Senegal; in **blue**, VZV isolated from serum samples from Senegal; in **green**, VZV isolated from nasopharyngeal swab samples from Senegal; and in **black**, VZV downloaded from NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/about/).

surveillance and targeted public health interventions. Given the overlapping clinical hallmarks and the potential for misdiagnosis, a syndromic surveillance approach that integrates molecular diagnostics is essential for effective outbreak response and patient management in endemic regions.

This study has several limitations that should be considered when interpreting the findings. First, the number of patients included was modest ($n = 103$) and unevenly distributed across 14 administrative regions of Senegal. This limited sample size reduces the statistical power to compare clinical symptoms between herpesvirus-positive and herpesvirus-negative patients, and the findings should therefore be interpreted with caution. Second, the study was conducted over a relatively short period (August to December 2024), which may not capture the full seasonal or interannual variation in herpesvirus circulation. Third, some sociodemographic and clinical variables (e.g., age, sex) were missing due to incomplete records. Although statistical testing ($p = 0.210$) suggested that the missing data were randomly distributed and unlikely to introduce systematic bias, they nonetheless reduced the completeness of the analyses and may have affected the precision of subgroup estimates.

In terms of methodology, lesion swabs are considered the optimal specimen type for *q*PCR detection of Mpox and herpesviruses, yet serum samples were also tested in this study; their lower diagnostic sensitivity may have influenced pathogen detection rates.

Future research should therefore include larger, longitudinal cohorts with more systematic clinical, epidemiological, and laboratory data collection, including HIV testing and antimicrobial resistance analysis, to strengthen the generalizability and robustness of these findings.

Materials and methods

Ethical statement

This study was conducted in the framework of the routine surveillance for Mpox in Senegal, in accordance with the national ethical guidelines established by the MoHSA for public health research in accordance with WHO recommendations for outbreak response investigations. The field activities were regularly supervised by the authorities of the Senegalese MoHSA. Given its nature as a secondary analysis of de-identified data and in accordance with Act No. 2009-17, the need for written informed consent was waived by the National Ethics Committee for Health Research in Senegal (CNERS) that approved this study's protocol which include a minimal risk. However, the research was carried out with adherence to the ethical principles of the Declaration of Helsinki. All samples were anonymized.

Mpox case definition

Cases were classified according to the MoHSA criteria aligned with the WHO (2023)[36] and CDC (2023) guidelines[41]. A suspected case was defined as an individual presenting within 21 days with unexplained acute skin rash, mucosal lesions, or lymphadenopathy accompanied by at least one systemic symptom (fever > 38.5 °C, headache, myalgia, back pain, or profound weakness) and a history of travel to an endemic country. A probable case met suspected case criteria with an epidemiological link to a confirmed or probable animal or case within 21 days. A confirmed case had laboratory-detected monkeypox virus DNA.

Sample collection

As part of the national Mpox surveillance program, prospective case identification and sample collection were conducted from August to December 2024 in response to the 2024 Mpox PHEIC. The present study constitutes a secondary analysis of de-identified surveillance data and residual samples. Patients with clinical features of Mpox[36] were recruited from sentinel health facilities distributed across the 14 administrative regions of Senegal (Fig. 1). These facilities included regional hospitals, district health centers, and selected primary care clinics that participate in national surveillance. Sample collection followed the national Mpox surveillance protocol established by the MoHSA. Swabs from the exudate of vesicular or pustular lesions and lesion crusts, were collected during the acute rash phase using sterile swabs and transported in approximately 2 mL of viral transport medium (VTM) at 2–8 °C. Nasopharyngeal swabs were collected using sterile swabs and immediately placed in 2 mL of VTM. Venous blood was drawn into serum separator tubes for serum collection. All specimens were labeled with unique identifiers, stored at 4 °C at the collection site, and transported under cold chain to the Institut Pasteur de Dakar (IPD) for first line testings. Infection was confirmed by virus detection in skin swab samples, and an etiology was considered probable if it was detected in other sample types.

Molecular testing

From the serum, skin, or nasopharyngeal swabs, 200 µL of sample was lysed to extract viral DNA using the QIAamp DNA mini kit, (Qiagen, Germany) according to the manufacturer's instructions. The purified nucleic acid was eluted in a final volume of 60 µL of molecular-grade water. *q*PCR for Mpox detection was done using the commercial kits Lyophilized 1-step RT-PCR Polymerase Mix with the Lightmix modularDx Monkeypox virus assay, according to the manufacturer's instructions (Roche, Tibmol Biol, Berlin, Germany). The same DNA extracts were tested for herpesviruses using the commercial kit Allplex™ Meningitis-V1 assay (Seegene, South Korea), a multiplex *q*PCR panel that includes Varicella-Zoster virus (VZV), herpes simplex virus type 1 (HSV1), herpes simplex virus type 2 (HSV2), human herpesvirus 6 (HHV6), human herpesvirus 7 (HHV7), cytomegalovirus (CMV), and Epstein-Barr virus (EBV). Every experiment included appropriate internal and external quality controls. Sera were tested for measles and rubella specific immunoglobulin M (IgM) antibodies using WHO-recommended ELISA kits (Enzygnost Anti-Measles-Virus/IgM from Dade Behring or Siemens, Erlangen, Germany) according to the fabricant's instructions.

Genomic sequencing

A total of 28 herpesvirus-positive samples with threshold cycles (Ct) < 29 was selected for sequencing to maximize the likelihood of obtaining sufficient viral DNA for high-quality genome recovery, as lower Ct values generally indicate higher viral loads. In addition, 57 samples that were negative for both monkeypox virus and herpesvirus by *q*PCR were included for metagenomic sequencing to explore other possible etiologies. Selection was based on sample quality indicators, including sufficient residual volume after diagnostic testing and optimal storage conditions.

Enrichment-based methods

The DNA was quantified with a Qubit 1X dsDNA HS Assay Kit version Q33231 (Thermo Fisher Scientific, Waltham, MA, USA), and 10 ng of DNA was used with the TWIST EF 2.0 sequencing protocol version DOC-001170 REV 4.0 (Twist Bioscience, South San Francisco, CA, USA). The purified DNA was fragmented into 400 bp segments, and the libraries were prepared via the TWIST Universal Adapter System-Truseq version 101,308 (Twist Bioscience, USA). The indexed libraries were purified via magnetic beads and amplified via PCR. After washing with magnetic beads, the libraries were quantified via Qubit and pooled into groups of 8 samples. The pooled libraries were enriched via two methods, one with enrichment probes and the other with blockers. After heating and cooling, the hybridization mixture was incubated at 70 °C for 16 h. The beads attached to the enriched libraries were successively subjected to three wash-hybridization cycles by binding buffer. The enriched libraries were then amplified *via* PCR *via* the equinox amplification mix and primers. The purified libraries were then quantified and normalized to 2 nM. The experiment was performed on the Illumina MiSeq instrument, and consensus genomes were generated via Chan Zuckerberg ID (https://czid.org/).

Metagenomics

In brief, random amplification of the DNA fragments was carried out via the sequence-independent single-primer amplification (SISPA) method, followed by quantification and enzymatic fragmentation (tagmentation) of the DNA. Libraries were then prepared via the Nextera XT Library Prep Kit (Illumina, San Diego, California, USA) according to the manufacturer's instructions. The libraries were labeled via the indices Nextera XT index kit V2 and pooled at the same concentration. The quality of the libraries was validated, and their concentration was standardized via a Qubit fluorometer (Invitrogen, Waltham, Massachusetts, USA) before sequencing. Whole-genome sequencing was performed with paired reads via the Illumina MiSeq v2 reagent kit (300 cycles) on an Illumina MiSeq instrument. Data analysis was performed via fully open-source EDGE bioinformatics software to generate consensus genomes.

Phylogenetic analysis

Our dataset included VZV sequences retrieved from GenBank and newly characterized sequences from patients presenting clinical features of Mpox in Senegal. After multiple alignments of our dataset were performed *via* the Alignment Program MAFFT version 7 (https://mafft.cbrc.jp/alignment/server/), a Bayesian analysis was performed to determine the most appropriate nucleotide substitution model, considering the lowest Bayesian information criterion (BIC) score. The maximum likelihood (ML) phylogenetic tree was inferred from 1000 replications via IQTree online software (http://iqtree.cibiv.univie.ac.at/) with the best-fit nucleotide substitution model to our dataset. The ML tree was rooted at the midpoint, and nodes were marked with bootstrap values.

Statistical analysis

We performed descriptive statistical analyses to summarize the population across age, sex, and other pathologies (measles, rubella, etc.) according to herpesvirus status. Categorical variables were compared between herpesvirus-positive and herpesvirus-negative groups using Fisher's exact test, and p-values < 0.05 were considered statistically significant. Temporal and geographical distributions were performed, as was the distribution of herpes cases by sex. Statistical analyses were performed via **RStudio software version 4.4.0**.

Data availability

All data have been added to this version of the manuscript. The datasets used in the current study are available from the corresponding author and will be made available on request.

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Author contributions

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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