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Epidemiological and genomic evolution of the ongoing outbreak of clade Ib mpox virus in the eastern Democratic Republic of the Congo

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In September 2023, an ongoing mpox outbreak emerged in South Kivu (Democratic Republic of the Congo) that spread to other regions and countries. Here we describe the epidemiological and genomic evolution of the outbreak between September 2023 and June 2024. Samples were collected from hospitalized patients, along with data on residence and possible exposures. Employee numbers and locations were recorded for bars with sex workers. Where possible, exposures were linked to genomic sequencing data for cluster analysis. In total, 670 cases were admitted to Kamituga General Referral Hospital from 17 health areas. Among the cases, 52.4% were in females and 47.6% in males. The majority (83.4%) were linked to professional sexual interactions. Seven deaths occurred, and three healthcare workers acquired mpox. Eight out of 14 pregnant women had fetal loss. Phylogenetic analysis revealed three clade Ib clusters. Longer branches of a sequence clustering with sequences from Kenya, Uganda, Sweden and Thailand indicate more undocumented spread. Mutations were mostly APOBEC3-type mutations indicative of sustained human-to-human transmission. No clear link between sequence cluster, bar or health area was observed. These data suggest rapid spread mostly through sexual contact within densely populated areas. The spread to neighboring countries highlights the need for extended cross-border collaboration, health education strategies focusing on sex workers, contact tracing, clinical care and surveillance.

Mpox is an emerging zoonotic disease caused by mpox virus (MPXV). The first recorded human case was in 1970 in a 9-month-old child in the Democratic Republic of the Congo (DRC)[1]. MPXV is historically enzootic in Western and Central Africa, and there have been sporadic mpox outbreaks in humans with increasing frequency in West and Central African countries[2]. The DRC is the sole country that continually reported mpox cases in the past five decades, but there was limited global interest in supporting containment efforts. This changed when a widespread outbreak of clade IIb MPXV occurred worldwide in May 2022 that was transmitted primarily through men who have sex with men[3–5]. Although previous mpox outbreaks in regions with enzootic MPXV reservoirs have suggested potential for spread to more diverse populations and through different modes of transmission, this has been very limited in the global outbreak.

Two genetically distinct clades of MPXV have been described. Clade I is thought to cause more severe disease with reported case

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fatality rates (CFRs) of up to 10.6% in suspected cases, whereas clade IIb is associated with milder symptoms and a lower CFR of roughly 0.1%. However, this is difficult to show with certainty owing to a lack of standardized case ascertainment[6,7].

In November 2023, the World Health Organization noted an unprecedented increase in the number of notified cases across the DRC (https://www.who.int/emergencies/disease-outbreak-news/item/2024-DON522). As of 5 January 2025, more than 9,500 laboratory confirmed cases have been notified with an estimated CFR of 3.4%. From genomic sequencing, it has become apparent that there are several ongoing outbreaks with different origins, age groups involved and suspected modes of transmission[8]. A particular concern has been the rapid increase of cases in South Kivu, since the initial detection in September 2023, with a shift in the epidemiological pattern compared with other regions[9]. Recently, we showed that this outbreak is associated with clade Ib viruses and that the majority of these cases are transmitted through (hetero)sexual contact[10].

Here, we provide a detailed overview of the outbreak in South Kivu from 29 September 2023 to the end of June 2024, including the mapping of cases, assessment of possible exposures and genomic sequencing to understand the evolution of the outbreak.

A total of 670 hospitalized mpox case records were listed as confirmed, probable or suspected (Extended Data Fig. 1a) between 29 September 2023 and 30 June 2024. In total, 646/670 (96.4%) were confirmed by polymerase chain reaction (PCR). Three cases were healthcare workers, and seven individuals died (1% CFR). In contrast to other outbreaks in the DRC, only 15.5% (104/670) of suspected cases were children under 16 years. Among these, 45 were less than 5 years of age. The majority were aged between 15 and 24 years. There were more female than male cases (351/670 (52.4%) versus 319/670 (47.6%)), and cases were reported from 17/23 health areas (Table 1). The Kamituga health zone reported peak levels up to 14 admissions per week in 2023, increasing to 42 admissions by week 25 of 2024 (Extended Data Fig. 1). The outbreak expanded to Bigombe, Luliba, Isopo, Kele Katutu, Ngambwa and Kankanga by weeks 7, 10, 11, 15 and 24, respectively. Nyangezi, near Rwanda and Burundi, saw a sharp increase in cases from week 14 in 2024 onward. Given this expansion, a few cases were sampled to include sequencing analysis of the Kamituga patient selection.

Four of the seven fatalities were young adults (20–30 years), with three females and one male patient. One patient had a human immunodeficiency virus (HIV) co-infection and died after developing neurological complications, and a second patient had complications from a cesarean section. A third person developed confluent lesions, with clinically suspected bacterial infection, and patient 4 died after developing respiratory distress. The other three recorded deaths were infants: one was born with lesions from intrauterine infection, and two babies were infected postnatally. In two cases, breast milk samples tested positive. HIV status was not known. At that time, there was no clinical characterization protocol in place and, therefore, further details were not available.

During the study period, 14 pregnant women were hospitalized, of whom 8 (57.1%, aged 16–29 years) suffered intrauterine fetal death and/or fetal loss, with visual evidence of infection (pox lesions) in one fetus and a PCR-positive placenta in another case. Five women lost their pregnancies in the first trimester and three in the second trimester. Three were housewives, two were professional sex workers (PSWs), one was a health worker, one was a trader and one reported no profession. No samples were obtained from aborted fetuses.

On 29 September 2023, the first case was reported in the Poudriere health area and linked to a bar visit. We interviewed hospitalized cases with a standardized case reporting form including assessing potential sexual exposures. Among the 670 hospitalized mpox cases, 83.4% (559/670) reported recent sexual contact in bars, of which 44.6% were female and 38.8% were male.

Table 1 | Epidemiological data of the ongoing mpox outbreak

Kamituga health areas	Population	Mpox cases	Relative frequency (%)	Sequenced samples (% total)	PSWs[a]	Bars
Kele Sidem	10,618	10	0.09		0	0
Asuku	11,002	73	0.66	8 (11.0%)	38	3
Bigombe	7,274	3	0.04		18	2
Isopo	5,403	1	0.02		0	0
Kabukungu	13,758	115	0.84	7 (6.1%)	152	10
Kalingi	12,888	28	0.22	4 (14.3%)	57	5
Kankanga	5,622	6	0.11	1 (16.7%)	0	0
Katunga	13,492	43	0.32	3 (7.0%)	7	1
Kele Katutu	11,234	3	0.03		21	0
Kibe	12,073	2	0.02		34	3
Kimbangu	16,132	105	0.65	11 (10.5%)	45	5
Luliba	2,870	11	0.38	1 (9.1%)	18	2
Mero	25,598	205	0.80	14 (6.8%)	161	13
Ngambwa	4,025	2	0.05		15	0
Poly Afia	12,105	13	0.11	3 (23.1%)	78	5
Poudriere	12,931	14	0.11		32	6
Soluluyu	10,921	36	0.33	4 (11.1%)	75	8
Total	**187,946**	**670**	**0.36**	**56 (8.4%)**	**751**	**63**

Description of number of inhabitants, bars, individuals employed as sex workers and notified mpox cases for the health areas in Kamituga that reported cases during the study period.
[a]Number of self-reported PSWs.

Following the initial observation of the contribution of sex workers driving transmission, and the mentioning of specific bars as possible places of exposure, we carried out a census of 63 bars and PSWs of Kamituga health zone and also inventoried the number of PSWs involved (n = 751) (Table 1 and Extended Data Fig. 2b). Overall, 200/751 (26.6%) of the PSWs were infected during the study period. We then mapped the population density of the Kamituga health zone, as well as the number of PSWs and reported mpox cases during the study period (Extended Data Fig. 2). In 2023, Mero had the highest population density, followed by Kimbangu and then by six health areas with similar numbers of people (Table 1 and Extended Data Fig. 2a). As expected, the expansion of the outbreak from September onward closely matched these in more populated health areas (Extended Data Fig. 2c).

We sequenced 58 MPXV genomes of 54 patients from samples during the current outbreak collected between October 2023 and May 2024. Phylogenetic analysis and inspection of the mutation patterns revealed three potential clusters and two potential subclusters consisting of sequences of different collection dates including April/May 2024, suggesting several different, ongoing, transmission chains (Fig. 1). Indicated by the colored tips, the cluster patterns did not directly relate to the health area origin of the patients. Cluster A shows many unique mutations suggesting undetected circulation or circulation outside the Kamituga health zone, also corroborated by the two samples in this cluster from Kamanyola (yellow tips). Cluster B shows few shared and unique mutations compared with the other clusters. This cluster shows several cases of miniclusters of two cases with identical sequences. Three of these miniclusters consisted of sequences from the same patient.

We observed an abundance of APOBEC3 mutations (28/35 linking mutations and 55/82 unique mutations) indicative of continuous human-to-human transmission. Most APOBEC3 mutations were observed in cluster A, which includes two sequences from cases from

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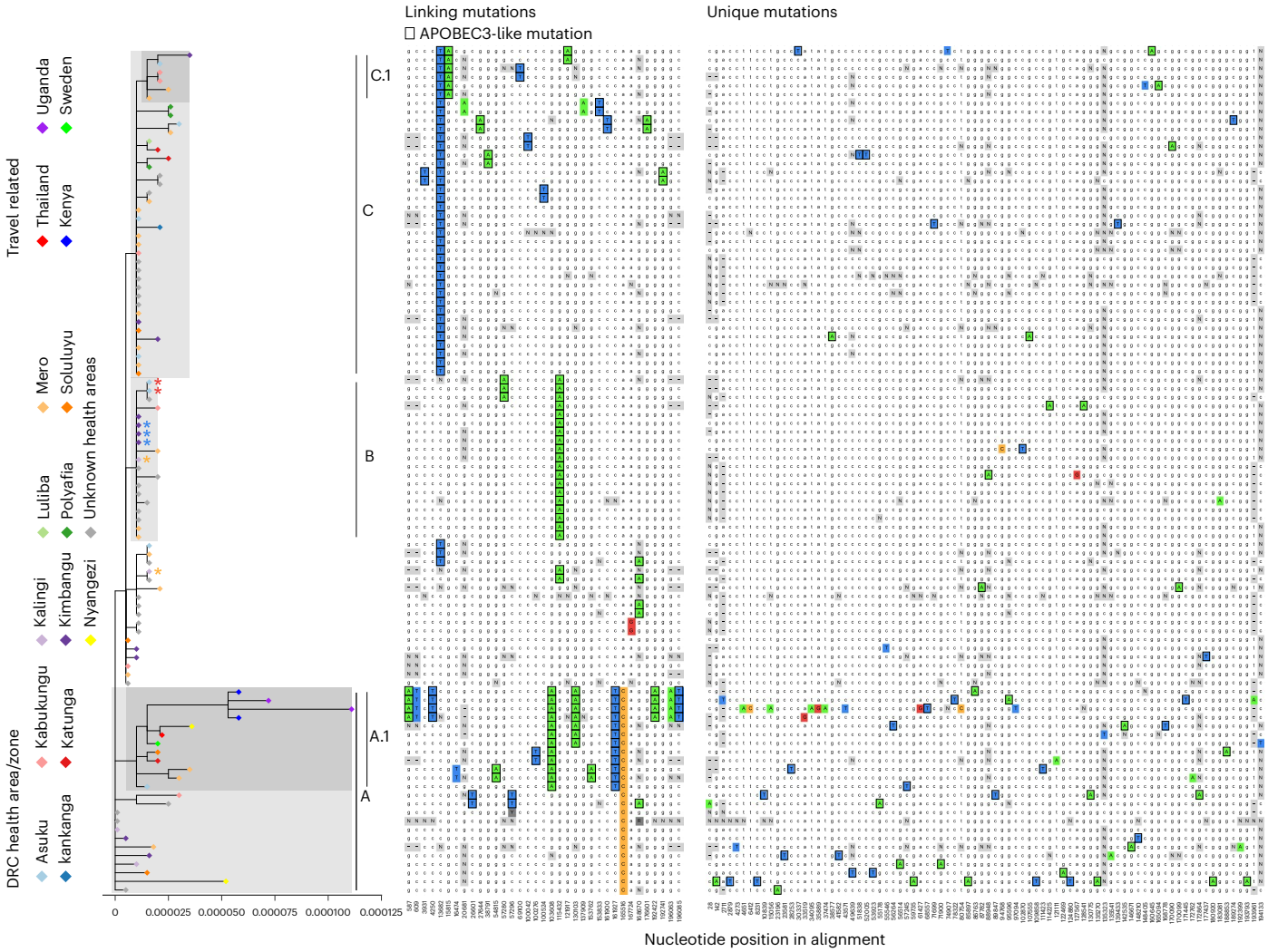

Fig. 1 | Phylogenetic analysis. Zoom-in of phylogenetic tree specific for the currently shared and newly sequenced clade Ib sequences. A phylogenetic tree with detailed tip labels is shown in Extended Data Fig. 3. Left: the phylogenetic tree with colored tips indicating the health area of the originating patient. The colored asterisks indicate sequences from different samples of the same patient. Proposed clusters are shown by shading and annotated by markings on the right side of the tree. Middle: linking mutations, present in two or more cases. Right: unique mutations. Differences from the majority rule consensus are highlighted in color. Mutations with characteristic APOBEC3 signature are marked with a black box. Positions that were masked by manual curation or due to too little coverage are indicated with 'N'. Ambiguous nucleotides are marked in dark gray.

Kamanyola. These two cases were not directly linked to each other and show up to ten unique mutations, suggesting considerable transmission before their detection. The sequences of travel-associated cases in Kenya, Tanzania, Thailand and Sweden are part of subcluster A.1 and show an additional seven linked mutations, of which five have the APOBEC3 signature. Six of the 7 mutations are situated in the inverted terminal repeat region effectively creating a reverse complement duplication of the mutations.

Few formal case investigations of clade I have been documented during outbreaks in Central Africa, and no sustained human-to-human transmission of clade I cases was reported until April 2023. Until the emergence of clade Ib in 2023, South Kivu Province had not reported continuous mpox cases, except for the limited sporadic cases in Shabunda (2012) and Minova (2014) health zones[10–12]. The data we have compiled here provide additional insights into the time trend and spatial distribution of the ongoing outbreak in South Kivu and shed light on the links of increasing mpox cases with population density and the presence of PSWs in bars within affected health areas. Health areas reporting continuous mpox cases were densely populated with numerous bars facilitating the local sex industry, largely tied to the region's gold-mining activities. Most patients reported bar contact before developing mpox-like symptoms. The Nyangezi health zone, with similar mining-related activities, reported the second-highest case numbers. Kamanyola City serves as a transit hub where travelers from remote mining areas often visit bars before crossing into Rwanda, Burundi or towns such as Bukavu and Goma. PSWs from these three countries also operate in Kamanyola, further heightening the risk of cross-border transmission. Notably, recent MPXV clade Ib cases have been reported in Rwanda, Kenya, Uganda and Burundi[13].

There currently is considerable uncertainty about properties associated with clade Ib regarding the transmissibility and severity of the disease. Based on animal studies and outbreak investigations, virulence differences have been observed between clade I and clade II infections[6]. However, epidemiological data is difficult to interpret with certainty, owing to the challenges in case ascertainment in low-income settings and the lack of systematic follow-up of cases in households[6]. In this study, a CFR of 1% was observed, which is probably an overestimate given that these were hospitalized patients. The CFR might also be influenced by the age range of cases; we observed three deaths among 45 children less than 5 years of age. In addition, the deletion of

the virulence gene D14L (OPG032) in clade Ib viruses, which is also not present in clade II viruses, might result in lower virulence[14,15]. Notably, among the first 170 confirmed clade Ib cases in Burundi, no death was reported[13]. Further studies are needed to elucidate this, along with the systemic integration of testing for HIV and other comorbidities.

A specific concern is the potential for complications of mpox in pregnant women. During this outbreak, 14 pregnant women were admitted at Kamituga General Referral Hospital, among whom 8 aborted, supporting the potential for intrauterine transmission of MPXV in pregnant people as also previously suggested[16,17]. In 2008, a MPXV clade I case of fetal death after placental infection was reported, which suggested risks of intrauterine infection, emphasizing that clinicians should be aware of this potential complication[17]. We also show the detection of MPXV DNA in breast milk, showing the potential of mother-to-child transmission during breastfeeding. However, the current knowledge precludes a separate assessment of the role of milk versus direct contact in transmission of MPXV from mothers to infants.

A further knowledge gap is the question of whether there are differences in transmissibility between MPXV clades and between clade Ia and clade Ib viruses. Although there is evidence for an increased rate of person-to-person spread in clade Ib infections, the opportunity for spread provided by specific risk behavior may also explain the observed pattern. The proportion of patients reporting recent exposure to a risk environment (bars or prostitution) did not decrease over the observation period, providing some evidence that the outbreak still is driven primarily by close sexual contact. A recent study described that, in children, pox lesions were mostly not detected in the genital area but more frequently elsewhere in the body, suggesting a role for other modes of transmission[18]. An animal study with clade IIb MPXV found an association between routes of exposure, viral load and rate of secondary transmission, with increased shedding and more transmission in animals infected by vaginal or rectal inoculation[19].

MPXV genome sequencing data were added to provide further insight into clade Ib dispersal. Several clusters and potential subclusters could be identified that can facilitate tracking the spread of the outbreak. Several APOBEC3-signature mutations were observed, particularly in cluster-A sequences. Based on currently available public data, all cross-border cases are linked to this cluster, suggesting sustained but undetected human-to-human transmission of clade Ib outside the Kamituga health zone before reaching Kamanyola.

In conclusion, we report an ongoing mpox outbreak thus far causing 7 deaths among 670 hospitalized individuals. Furthermore, 8/14 pregnant women infected with MPXV aborted. The outbreak appears to be driven by sexual activity with PSWs linked to bars, supporting the previously undocumented model of heterosexual transmission. Genomic analysis shows evidence for considerable underdetection. Cross-border surveillance, contact tracing, potential vaccination, access to rapid diagnostics and treatment are crucial to control further dispersal.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-025-03582-1.

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Methods

Ethical approval

The ethical clearance to conduct these studies was obtained from the Ethical Review Committee of the Catholic University of Bukavu (UCB/CIES/NC/022/2023).

Study design and participants

We collected demographic data and exposure histories of all individuals with MPXV infection symptoms admitted at Kamituga General Hospital between September 2023 and June 2024 who agreed to participate in the study. For consenting patients, geolocations were collected from the notification database in the health zones of South Kivu Province (https://data.humdata.org/dataset/drc-health-data). The Kamituga health zone contains 23 health areas within an area of 2,482 km^2. Kamituga General Referral Hospital serves as the central hub for these areas. Due to degraded infrastructure and scattered populations in rural areas, it can take up to 2 days to travel 60 km. Ethical clearance to conduct these studies was obtained from the Ethical Review Committee of the Catholic University of Bukavu (UCB/CIES/NC/022/2023). All study participants were introduced to the observational study and given the option to participate by providing informed consent or through parental permission. Patient information was anonymized. Patient care was provided by Kamituga health zone with the support of the national ministry of health. The hospital staff was trained in standard operating procedures for data collection, and the mpox treatment center was built far from the hospital facility.

Study area

Kamituga is South Kivu's largest gold-mining city located in the territory of Mwenga, part of South Kivu Province. South Kivu Province borders in the north with North Kivu Province, south and west with Maniema Province and south with Tanganyika Province. To the east, South Kivu borders Rwanda, Burundi and Tanzania. The city of Kamituga has more than 241,642 inhabitants (based on the 2024 Kamituga Health Zone report), of whom ~20,000 are employed in the mining industry. The remaining residents depend directly or indirectly on artisanal and small-scale gold mining for their livelihood.

Procedures and sample collection

Routine data, including age, gender, professional occupation, clinical symptoms, geolocations of mpox cases, concomitant presence of sexually transmitted infections, and comorbidities, were collected from patient records (or hospital investigation forms) and entered into a secured, anonymized database. The study team was informed about the objectives of the study, the consent process and the importance of standardized reporting.

Admission to Kamituga General Referral Hospital was based upon clinical diagnosis of human MPXV infection by hospital staff. A confirmed MPXV case was defined as an individual with laboratory-confirmed infection that was tested in the National Institute for Biomedical for Research. A case was listed as 'suspected' if a patient had an acute illness with fever, intense headache, myalgia and back pain, followed by 1–3 days of a progressively developing rash often starting on the face and spreading on the body. Finally, a case was listed as 'probable' if it satisfied the clinical definitions of suspected cases and had an epidemiological link to a confirmed or probable case but was not laboratory confirmed. A skin lesion was defined as a single circumscribed area and included presentations comparable to papules, pustules, fluid-filled vesicles or eschars.

Our team was prepared to carry out research in remote areas and be local field based since the beginning of the outbreak. Among the physicians were clinician psychologists who had experience working with similar people. To work with sex workers, clinical psychologists were consulted for training of the outbreak investigation team. This involved psychosocial epidemiology techniques, psychotherapy techniques enabling the team to build confidence with PSWs by avoiding disease stigmatization during the interviews.

Mapping of mpox cases

Geographic and epidemiological data were processed using the R programming language. The epidemiological curve was plotted using the ggplot2 package[20]. For the geographical maps, cases and sex workers were scaled by population density and aggregated by health area defined by the shapefiles provided by the national health ministry in the DRC available at https://data.humdata.org/dataset/drc-health-data. Map plots were made by using the sf and ggmap R packages[20,21].

Whole-genome sequencing

A total of 92 samples from 88 patients covering the entire time span of the ongoing mpox outbreak in the South Kivu region between September 2023 and May 2024 were selected for genomic investigations. Nucleic acids were extracted using the High Pure Viral Nucleic Acid Kit (Roche) (catalog no. 11858874001). A clade-Ib-specific real-time PCR[22], in combination with the US Centers for Disease Control and Prevention generic MPXV real-time PCR[23], was performed. Sixty-three samples with a Ct value below 30 were selected for whole-genome sequencing. Two different amplicon-based approaches were utilized to ensure a high sequencing depth and coverage (mpxv/rigshospitalet/2500/v1.0.0)[24]. Lysis buffer was used as the negative control. Amplicon-based sequencing libraries were generated as described previously[25]. Sequencing libraries were generated using the Native Barcoding Kit 24 v14 (SQK-NBD114.24) (ONT) and sequenced on a R10.4.1 flowcell (FLO-MIN114) (ONT). Reads were basecalled with high accuracy with Dorado Basecalling Server v.7.2.13 (ONT).

Data analysis and generation of sequences

The code used for data analysis is available via GitHub at https://github.com/dnieuw/mpox-south-kivu-mapping-manuscript. In brief, reads were trimmed using fastp v0.23.2 (https://github.com/OpenGene/fastp) and cutadapt (https://github.com/marcelm/cutadapt). Amplicon primers were trimmed with Ampliclip v1 (https://github.com/dnieuw/Ampliclip) and mapped to the reference genome NC_003310.1. Consensus sequences were generated using Samtools v1.10. Sequences with >85% genome coverage (n = 58) were included in the phylogenetic analysis.

All available clade I sequences on the National Center for Biotechnology Information and the Global Initiative on Sharing All Influenza Data (GISAID) on 31 August 2024 were merged, and a maximum likelihood phylogenetic tree was generated using IQ-Tree v2.2.0.3 using the K3Pu+F+I model as the best predicted model (see GISAID acknowledgment in Extended Data Table 1). The maximum likelihood phylogenetic tree was visualized using a custom R script making use of mainly the ggtree, tidytree, ape and patchwork packages. APOBEC3 mutations were identified as described by O'Toole and colleagues[26]. Based on the tree and the specific mutations, we identified clusters with arbitrarily assigned numbers that were subsequently added as a field to the metadata file for testing of possible associations with potential exposure routes (specific bars, households and so on).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Consensus sequences are available on GenBank under the accession number PQ305763-PQ30582 and GISAID under the accession ID ENA under the accession numbers EPI_ISL_18886301, EPI_ISL_18886467, EPI_ISL_18886588, EPI_ISL_18886634, EPI_ISL_18886635, EPI_ISL_18886639, EPI_ISL_19357138, EPI_ISL_19357623, EPI_ISL_19357654, EPI_ISL_19361815, EPI_ISL_19361896, EPI_ISL_19361897, EPI_ISL_19363685,

EPI_ISL_19363686, EPI_ISL_19364035, EPI_ISL_19364149, EPI_ISL_19364369, EPI_ISL_19364511, EPI_ISL_19364948, EPI_ISL_19365441, EPI_ISL_19365466, EPI_ISL_19365506, EPI_ISL_19365507, EPI_ISL_19365542, EPI_ISL_19365543, EPI_ISL_19365544, EPI_ISL_19365545, EPI_ISL_19366314, EPI_ISL_19367803, EPI_ISL_19381268, EPI_ISL_19382151, EPI_ISL_19382354, EPI_ISL_19382355, EPI_ISL_19382807, EPI_ISL_19382808, EPI_ISL_19382908, EPI_ISL_19382942, EPI_ISL_19382949, EPI_ISL_19382965, EPI_ISL_19422968, EPI_ISL_19422969, EPI_ISL_19429464, EPI_ISL_19429465, EPI_ISL_19429466, EPI_ISL_19431759, EPI_ISL_19431760, EPI_ISL_19431761-63, EPI_ISL_19433147, EPI_ISL_19434036-38, EPI_ISL_19434063, EPI_ISL_19434064, EPI_ISL_19439694-97, EPI_ISL_19460816, EPI_ISL_19460883, EPI_ISL_19460954, EPI_ISL_19461017, EPI_ISL_19462380 and EPI_ISL_19462381.

Code availability

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Author contributions

All authors approved the final version of the paper. L.M.M., J.C.U., P.N. and M.K. conceptualized and designed the study. L.M.M., B.B.O.M., L.S., M.B., M.K., F.M.A., S.O. and D.F.N. contributed to data acquisition, L.M.M., B.B.O.M., L.S., D.N. and M.K. drafted the paper and figures. L.M.M., J.B.M. and F.B.S. collected epidemiological data and arranged for patient sampling. All authors were involved in paper writing and approved the final version.

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-025-03582-1.

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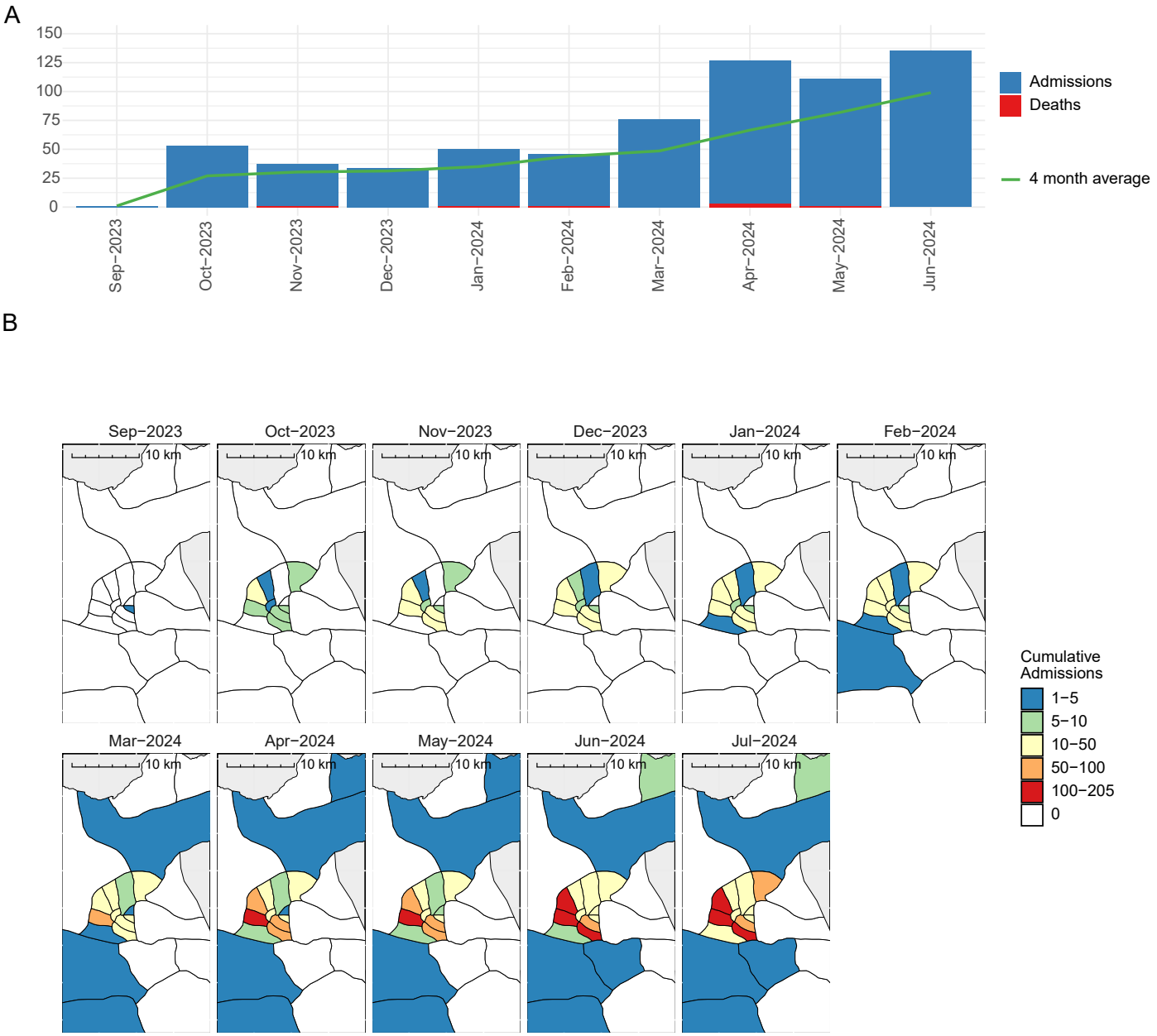

Extended Data Fig. 1 | Epicurve and geographic spread of admissions. **(A)** Epicurve of outbreak based on Kamituga health zone surveillance dataset for the mpox patients' admissions at Kamituga hospital and the deaths per epidemiological week between September 29th, 2023 to June 29th, 2024. **(B)** Map showing evolution of the outbreak in time and space within health areas of Kamituga health zone. Black dots indicate in what epidemiological week and health area a first complete genome sequence was generated. Grayed-out areas are outside of Kamituga health zone, the others represent all health areas in Kamituga health zone. Contains information from the Humanitarian Data Exchange, which is made available under the Open Database License (ODbL).

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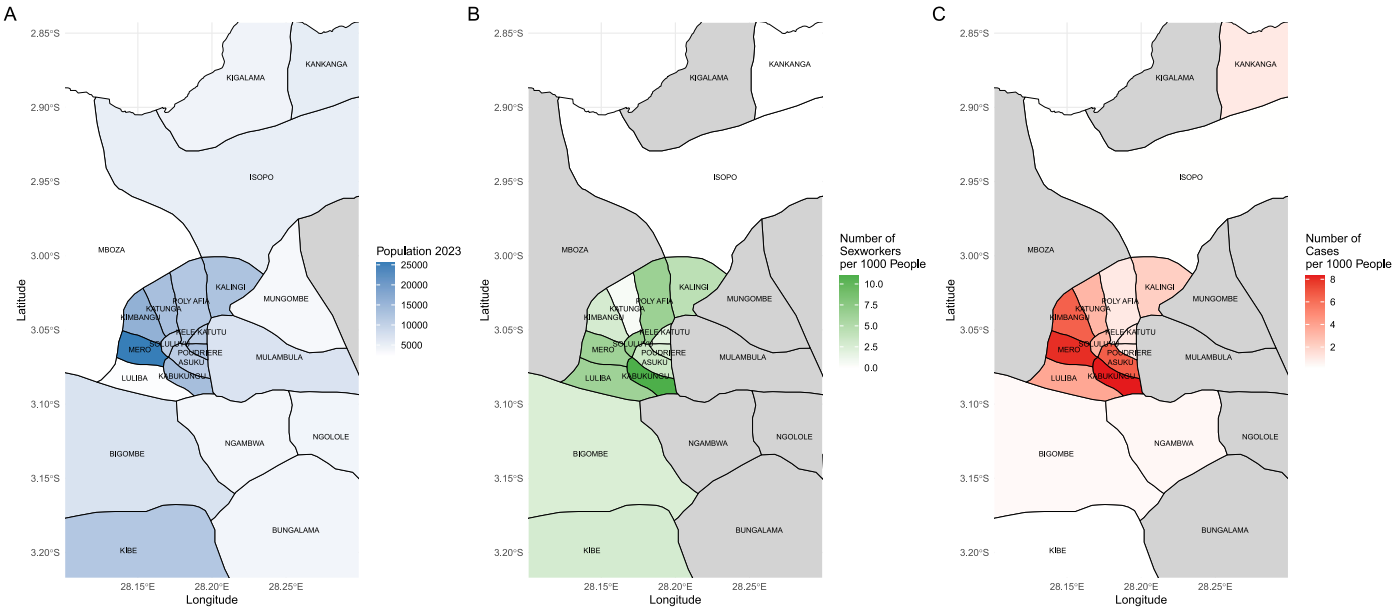
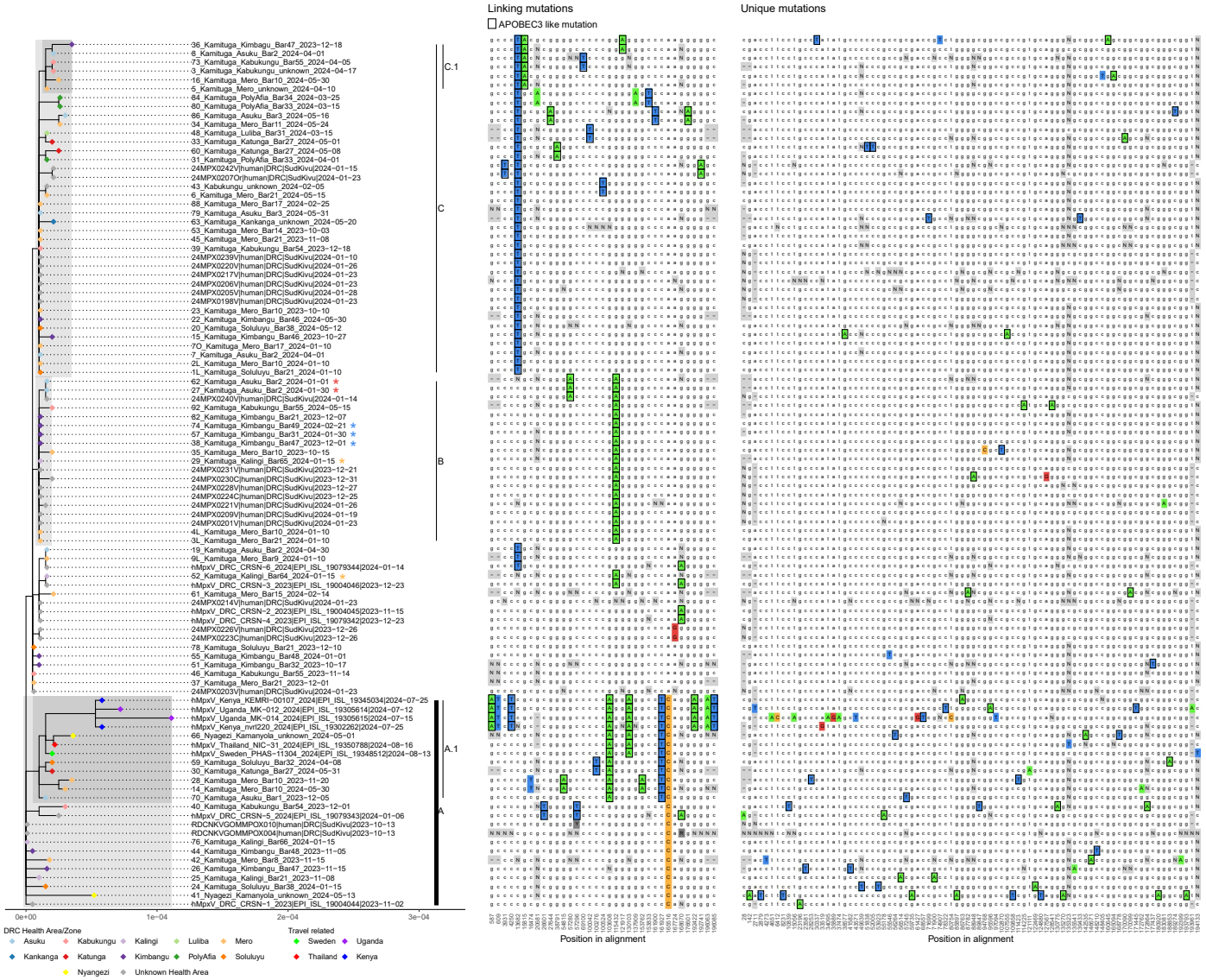


Extended Data Fig. 2 | Population, sex worker, and case counts. (A) reports population density (2023) per health area in Kamituga health zone, between September 29th, 2023 and April 21st, 2024, South-Kivu, DRC, **(B)** reports number of sex workers per 1000 inhabitants, **(C)** reports number of mpox cases per 1000 inhabitants. Gray areas do not have any recorded population (A), sex workers (B), or have not reported any cases **(C)**. Contains information from the Humanitarian Data Exchange, which is made available under the Open Database License (ODbL).

https://doi.org/10.1038/s41591-025-03582-1



Extended Data Fig. 3 | Zoomed in phylogeny and mutations. Zoom-in of phylogenetic tree with detailed tip labels specific for the currently shared and newly sequenced Clade Ib sequences. Left panel shows the phylogenetic tree with colored tips indicating the health area of the originating patient. Colored asterisks indicate sequences from different samples of the same patient. Proposed clusters are shown by shading and annotated by markings on the right side of the tree. Middle panel shows linking mutations, present in two or more cases. Right panel shows unique mutations. Differences from the majority rule consensus are highlighted in color. Mutations with characteristic APOBEC3 signature are marked with a black box. Positions that were masked by manual curation or due to too little coverage are indicated with 'N'. Ambiguous nucleotides are marked in dark gray.

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Extended Data Table 1 | GISAID EPI numbers and acknowledgements

Accession ID	Originating Laboratory	Submitting Laboratory	Authors
EPI_ISL_19004044	Centre de Recherche en Sciences Naturelles de Lwiro	Centre de Recherche en Sciences Naturelles de Lwiro	Leandre Murhula Masirika, Jean Claude Udahemuka, Pacifique Ndishimye, Gustavo Sganzerla Martinez, Patricia Kelvin, Mallyamungu Bubala Nadine, Bilembo Kitwanda Steeven, Franklin Kumbana Mweshi, Léandre Mutimbwa Mambo, Bas B. Oude Munnink, Justin Bengehya Mbiribindi, Freddy Belesi Siangoli, Trudie Lang, Jean M. Malekani, Frank M. Aarestrup, Marion Koopmans, Leonard Schuele, Jean Pierre Musabyimana, Brigitte Umutoni, Ali Toloue, Benjamin Hewins, Mansi Dutt, Anuj Kumar, Alyson A. Kelvin, Jean-Paul Kabemba Lukusa, Christian Gortazar, David J Kelvin, Luis Flores
EPI_ISL_19004045, EPI_ISL_19004046	Centre de Recherche en Sciences Naturelles de Lwiro	Centre de Recherche en Sciences Naturelles de Lwiro	Leandre Murhula Masirika, Jean Claude Udahemuka, Pacifique Ndishimye, Gustavo Sganzerla Martinez, Patricia Kelvin, Maliyamungu Bubala Nadine, Bilembo Kitwanda Steven, Franklin Kumbana Mweshi, Léandre Mutimbwa Mambo, Bas B. Oude Munnink, Justin Bengehya Mbiribindi, Freddy Belesi Siangoli, Trudie Lang, Jean M. Malekani, Frank M. Aarestrup, Marion Koopmans, Leonard Schuele, Jean Pierre Musabyimana, Brigitte Umutoni, Ali Toloue, Benjamin Hewins, Mansi Dutt, Anuj Kumar, Alyson A. Kelvin, Jean-Paul Kabemba Lukusa, Christian Gortazar, David J Kelvin, Luis Flores
EPI_ISL_19079342, EPI_ISL_19079343, EPI_ISL_19079344	Centre de Recherche en Sciences Naturelles de Lwiro (CRSN Lwiro)	Centre de Recherche en Sciences Naturelles de Lwiro (CRSN Lwiro)	Leandre M Masirika, Anuj Kumar, Mansi Dutt, Ali Toloue Ostadgavahi, Benjamin Hewins, Maliyamungu B Nadine, Bilembo K Steven, Franklin K Mweshi, Léandre M Mambo, Justin B Mbiribindi, Freddy B Siangoli, Alyson A Kelvin, Jean Claude Udahemuka, Patricia Kelvin, Luis Flores, David J Kelvin, Gustavo Sganzerla Martinez
EPI_ISL_19302262	National Public Health Laboratory - NVRL	Kenya Medical Research Institute (KEMRI) / Walter Reed Army Institute of Research - Africa	Gathil Kimita, Joseph K. Kaingu, George O. Awinda, Allan P. Lemtudo, Esther A. Omuseni, Josphat N. Nyataya, Beth K. Mutai, John N. Waitumbi
EPI_ISL_19305614, EPI_ISL_19305615	MRC/UVRI & LSHTM Uganda Research Unit, Uganda Virus Research Institute	MRC/UVRI & LSHTM Uganda Research Unit, Uganda Virus Research Institute	Nicholas Bbosa, Stella E. Nabirye, Hamidah S. Namagembe, Ronald Kiiza, Alfred Ssekagiri, Mary Munyagwa, Arafat Bwambale, Stephen Bagonza, Henry Kyobe Bosa, Mary Rodgers, Francisco Averhoff, Michael Berg, Robert Downing, Gavin Cloherty, Julius Lutwama, Pontiano Kaleebu, Deogratius Ssemwanga
EPI_ISL_19345034	Arbovirology/Viral Haemorrhagic Fever Lab, Kenya Medical Research Institute (KEMRI)	Arbovirology/Viral Haemorrhagic Fever Lab, Kenya Medical Research Institute (KEMRI)	Langat,S., Nyunja,A., Pilarowski,G., Okunga,E., Ofula,V., Oluniyi,P., Koskei,E., Koka,H., Owaka,S., Chepkorir,E., Lutomiah,J., Langat,D., Khamadi,S., Limbaso,K.
EPI_ISL_19348512	The Public Health Agency of Sweden	The Public Health Agency of Sweden	Oskar Karlsson Lindsjö, Maria Lind Karlberg, Klara Sondén
EPI_ISL_19350788	Hospital in Bangkok	Department of Medical Sciences, National Institute of Health	Pilailuk Okada, Siripaporn Phuygun, Nuttida Thongpramul, Titichaya Mebuathong, Pakorn Piromtong, Thanutsapa Thanadachakul, Thitipong Yingyong, Archawin Rojanawiwat, Ballang Uppapong, Yongyos Thammavuth

Additionally, GenBank accession numbers PP601207.1 - PP601228.1 and the 58 MPXV Clade Ib sequences generated in this study were used for phylogenetic analysis.

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Software and code

Policy information about availability of computer code

Data collection

The basic descriptive data was collected from the hospital database, and in the provincial surveillance database. Geographic and epidemiological data were processed using the R programming language. For the geographical maps cases and sexworkers were scaled by population density and aggregated by health area defined by the shapefiles provided by national health ministry in Democratic Republic of the Congo available online: https://data.humdata.org/dataset/drc-health-data. .

Data analysis

Mapping of mpox cases
Geographic and epidemiological data were processed using the R programming language. The epidemiological curve was plotted using the "ggplot2" package (Pebesma, 2018). For the geographical maps cases and sexworkers were scaled by population density and aggregated by health area defined by the shapefiles provided by national health ministry in Democratic Republic of the Congo available online: https://data.humdata.org/dataset/drc-health-data. Map plots were made by using the "sf" and "ggmap" R packages (Pebesma, 2018; Kahle and Wickham, 2013).
Data analysis and generation of sequences
The code used for data analysis is available on GitHub: https://github.com/dnieuw/mpox-south-kivu-mapping-manuscript. Briefly, reads were trimmed using fastp v0.23.2 (https://github.com/OpenGene/fastp) and cutadapt (https://github.com/marcelm/cutadapt). Amplicon primers were trimmed with Ampliclip v1 (https://github.com/dnieuw/Ampliclip) and mapped to the reference genome NC_003310.1. Consensus sequences were generated using Samtools v1.10. Sequences with >85% genome coverage (n=58) were included in the phylogenetic analysis.

All available Clade I sequences on NCBI and GISAID on the 31st of August 2024 were merged and a maximum likelihood phylogenetic tree was generated using IQ-Tree v2.2.0.3 using the K3Pu+F+I model as best predicted model (GISAID acknowledgment is present in Extended Data Table 1). The maximum likelihood phylogenetic tree was visualized using a custom R script making use of mainly the ggtree, tidytree, ape, and

patchwork packages. APOBEC3 mutations were identified as described by O'Toole and colleagues (27). Based on the tree and the specific mutations, we identified clusters with arbitrarily assigned numbers that were subsequently added as a field to the metadata file for testing of possible associations with potential exposure routes (specific bars, households, etc).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

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Consensus sequences are available on GenBank under the accession numbers: PQ305763-PQ30582 and GISAID under the accession ID: ENA under the accession numbers: EPI_ISL_18886301, EPI_ISL_18886467, EPI_ISL_18886588, EPI_ISL_18886634, EPI_ISL_18886635, EPI_ISL_18886639, EPI_ISL_19357138, EPI_ISL_19357623, EPI_ISL_19357654, EPI_ISL_19361815, EPI_ISL_19361896, EPI_ISL_19361897, EPI_ISL_19363685, EPI_ISL_19363686, EPI_ISL_19364035, EPI_ISL_19364149, EPI_ISL_19364369, EPI_ISL_19364511, EPI_ISL_19364948, EPI_ISL_19365441, EPI_ISL_19365466, EPI_ISL_19365506, EPI_ISL_19365507, EPI_ISL_19365542, EPI_ISL_19365543, EPI_ISL_19365544, EPI_ISL_19365545, EPI_ISL_19366314, EPI_ISL_19367803, EPI_ISL_19381268, EPI_ISL_19382151, EPI_ISL_19382354, EPI_ISL_19382355, EPI_ISL_19382807, EPI_ISL_19382808, EPI_ISL_19382908, EPI_ISL_19382942, EPI_ISL_19382949, EPI_ISL_19382965, EPI_ISL_19422968, EPI_ISL_19422969, EPI_ISL_19429464, EPI_ISL_19429465, EPI_ISL_19429466, EPI_ISL_19431759, EPI_ISL_19431760, EPI_ISL_19431761-63, EPI_ISL_19433147, EPI_ISL_19434036-38, EPI_ISL_19434063, EPI_ISL_19434064, EPI_ISL_19439694-97, EPI_ISL_19460816, EPI_ISL_19460883, EPI_ISL_19460954, EPI_ISL_19461017, EPI_ISL_19462380, EPI_ISL_19462381.
Reference genome: NC_003310.1

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	The majority of patients were aged between 15 -24 years old. In total, 646/670 (96.1%) were confirmed by PCR. There were slightly more female than male cases (351/670 [52,4%] versus 319/670, [47,6%]. In contrast to other outbreaks in the DRC, only 15,5 % (104/670) of suspected cases were children under 16 years of age. Of these, 45 were less than 5 years old. Four of the seven fatalities were young adults (between 20 and 30 years), with three females and one male patient
Population characteristics	A total of 670 hospitalized mpox case records were listed as confirmed, probable, or suspected (Figure 1A) between September 29th, 2023 and June 30th, 2024. Three of these were health care workers, and seven deaths occurred during the study period (1%). In contrast to other outbreaks in the DRC, only 15,5 % (104/670) of suspected cases were children under 15 years of age. Of these, 45 were less than 5 years old. The majority of patients were aged between 16 -26 years old. In total, 646/670 (96.1%) were confirmed by PCR. There were slightly more female than male cases (351/670 [52,4%] versus 319/670, [47,6%], and cases were reported from 17 of the 23 health areas (Table I).Of the 670 hospitalized mpox cases, 83.4% (559/670) reported recent sexual contact in bars among which 44,6 % were female and 38.8% were male. Only a few cases reported to not have had sexual contact in bars: in total 16.6% (111/670) with 8.8% (59/670) males and 7.8 % (52/670) females.
Recruitment	Basic descriptive data as part of routine public health surveillance was collected for all patients hospitalised during the study period,. For patients who consented, additional information on possible exposures was collected.
Ethics oversight	The ethical clearance to conduct these studies was obtained from the Ethical Review Committee of the Catholic University of Bukavu (Number UCB/CIES/NC/022/2023.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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Life sciences study design

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Sample size	The study included all consenting patients admitted to a hospital in South Kivu province. A total of 670 hospitalized mpox case records were listed as confirmed, probable, or suspected between September 29th, 2023 and June 30th, 2024. Following the initial observation of the contribution of sex workers driving transmission, and the mentioning of specific bars as possible places of exposure, we carried out a census of bars and professional sex workers (PSW) of Kamituga health zone.
Data exclusions	No data were excluded
Replication	The first author worked in the provincial public health office to validate the findings.
Randomization	Not applicable
Blinding	Not applicable

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We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

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Methods

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