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Epidemiological and Clinical Profile of Mpox Diagnosed at the National Laboratory of Clinical Biology of Public Health in Bangui, Central African Republic

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Abstract

Introduction: Monkeypox (MPXV) epidemics historically result from the zoonotic spread of clade I monkeypox virus (MPXV) in Central Africa and clade II MPXV in West Africa. In 2022, subclade IIb caused a global epidemic linked to sexual transmission. It is in this context that this study was conducted to determine the prevalence of MPXV at the Bangui National Laboratory. **Methodology:** This was a prospective, multicenter analytical study. Skin, blood, and oropharyngeal swabs were collected from suspected MPXV patients. A total of 50 samples were recorded during this study. Diagnosis was performed using real-time PCR. The study received approval from the Research Ethics Committee of the Doctoral School of Human and Veterinary Health Sciences, University of Bangui (ESP/CE/78/2024). Participants gave verbal consent for data collection. **Results:** The mean age of patients was 29.20 years with a standard deviation of ±19.07 and the median age of individuals was 25.50 years with a male predominance of 22.73%. Out of 50 patients suspected of Mpox, 30% of patients had muscle pain followed by intense fever with lymphadenopathy and 20% were confirmed positive by polymerase chain reaction. The most dominant age group was between 11 and 20 years with a rate of 0.30% of positive cases for Mpox. The difference in patients infected with Mpox is not significant with a $p \leq 0.70$ and the male gender is the most dominant with a rate of 22.73% of cases of positivity to Mpox with a p-value of 0.46. **Conclusion:** This

study shows that molecular diagnostics identified mutations characteristic of clade I, with a strong signal of APOBEC3 mutation pressure, suggesting active human-to-human transmission in CAR. Enhanced surveillance and an early diagnostic strategy would be necessary to eradicate the Mpox epidemic in CAR.

Keywords

Mpox, RT-PCR, Clade I, Central African Republic

1. Introduction

Monkeypox (MPXV) epidemics historically resulted from the zoonotic spread of clade I monkeypox virus (MPXV) in Central Africa and clade II MPXV in West Africa [1] [2]. However, since 2022, a global MPXV epidemic has occurred, with 109,699 laboratory-confirmed cases and 236 deaths in 123 countries from January 2022 to September 2024. This epidemic was observed much more frequently in African countries [3] [4]. In response, the World Health Organization (WHO) declared a Public Health Emergency of International Concern [5]. MPXV is an enveloped double-stranded DNA virus of the Poxviridae family, which includes variola (the causative agent of monkeypox) and vaccinia (used in smallpox vaccination) viruses. Human-to-human transmission occurs primarily through direct contact with skin lesions, bodily fluids, contaminated objects, or respiratory droplets from an infected person. Animals, particularly rodents, play a crucial role in zoonotic transmission. In humans, MPXV causes Mpox, characterized by fever, lymphadenopathy, and a vesiculopapular rash. There are two distinct genetic clades of MPXV: clade I, predominantly present in Central Africa, particularly Central African Republic (CAR), and is associated with severe clinical symptoms and significant mortality (4% - 11%), while clade II, largely confined to West Africa until the 2022 global epidemic, causes milder disease and a lower mortality rate of <4% [2].

Historically, clade I MPXV has predominated, accounting for 95% of reported cases. In 2017, a major outbreak of clade IIb MPXV occurred in Nigeria, with sustained human-to-human transmission, including through sexual contact. These findings were overlooked until a clade IIb lineage—B.1 spawned a global epidemic in May 2022, with 95,226 confirmed cases in 117 countries by March 2024 [4]. Genomic analyses of B.1 revealed a mutational pattern suggesting non-canonical evolution, mediated by an apolipoprotein B messenger RNA editing enzyme, cytosine deamination of the catalytic subunit 3 (APOBEC3), a hallmark of MPXV human-to-human transmission [5] [6]. MPXV mutations were confirmed in vitro to originate from the apolipoprotein B mRNA editing enzyme, catalytic polypeptide type 3F (APOBEC3F), suggesting that clade IIb entered human populations as early as 2015 [7] [8]. By April 2024, the global outbreak of clade IIb B.1 had largely subsided, although the virus continued to circulate in Nigeria and other countries. On July 20, 2024, Central African Republic declared

an outbreak of Mpox on its territory. It is in this context that this study was conducted to determine the prevalence of Mpox at the Bangui National Laboratory.

2. Methodology

Type and Location of Study

This was a prospective analytical study conducted over a 6-month period, from January 2025 to June 2025, at the National Laboratory of Clinical Biology and Public Health (LNBCSP). Sampling was comprehensive and included all blood, lesion, and scab samples collected from patients during Mpox virus investigations in Central African Republic.

3. Inclusion and Exclusion Criteria

The study included samples confirmed positive for Mpox by PCR at the National Laboratory, collected during the study period, and associated with a complete clinical record (age, sex, symptoms, and origin). Negative or unconfirmed samples, those with incomplete clinical data, and samples that did not comply with ethical guidelines were excluded.

3.1. Sampling and Data Collection

In January 2025, a multidisciplinary team composed of representatives from the Ministry of Health, the LNBCSP, and health districts conducted an investigation into the Mpox epidemic in the epidemic-affected health districts of the Central African Republic. A standardized form containing sociodemographic characteristics (age, sex, place of residence, occupation, and nationality), clinical signs, sample type, and collection date was used. Interviews were conducted with individuals suspected of having MPOX, their parents, and healthcare professionals.

3.2. Sample Collection and Diagnosis

Oropharyngeal swabs, blood, and skin lesion samples were collected for the diagnosis of MPOX.

3.2.1. Genomic DNA Extraction

The QIAampR Viral DNA Mini (250) Kit Cat. No. 52906 (USA) was used for DNA extraction. The Qiagen protocol follows the four main steps to follow during DNA extraction and purification. An additional step to aid viral cell wall lysis was added compared to the manufacturer's instructions.

- **Viral Lysis**

This step aims to destroy the viral cell wall to make the DNA accessible. 200 µL of AL (lysis) buffer and 20 µL of proteinase K are added and vortexed for 15 seconds, then incubated at 56°C in a water bath for 10 minutes.

- **DNA Precipitation**

This step aims to separate the DNA from other viral components such as proteins, lipids, and carbohydrates. To do this, 200 µL of 95% ethanol was added to

the mixture, which was vortexed, and then centrifuged. The role of the ethanol is to precipitate the DNA in the cold. The mixture was then transferred to a Qiagen column, inserted into a 2 ml collection tube, and centrifuged at 8000 rpm for 1 minute.

- **Washing**

An initial wash was performed using 500 µL of Wash Buffer 1 (AW1) introduced into a Qiagen column, which was then centrifuged for 1 minute at 8000 rpm. The QIAamp column was placed in a new 2 ml collection tube. The filtrate was then poured. A volume of 500 µL was added to a second Wash Buffer (AW2) and centrifuged at 14,000 rpm for 1 minute to remove the ethanol.

- **Elution**

After washing, the Qiagen column was transferred to a 1.5 ml tube for DNA collection. A volume of 100 µL of Elution Buffer (AVE) was added to the column and incubated at room temperature for one minute, then centrifuged at 8000 rpm. The DNA contained in the tube was then stored at −20˚C until use.

3.2.2. Preparation of the Reaction Mixture

Two separate rooms with biosafety hoods were prepared, one for the reaction mixture (clean room) and the other for the addition of DNA (dirty room).

In the clean room. The dilution of the reaction mixture was carried out in two steps:

- Solution A of the positive control was prepared in a 2 ml Eppendorf tube: 98 µL of Taq Path Control Dilution Buffer was taken and placed in the Eppendorf tube. 2 µL of Taq Path Positive was taken and added to the same tube, then mixed.

- Solution B is a ready-to-use solution.

In a 2 ml Eppendorf tube, withdraw 87.5 µL of solution B and place in the tube, then withdraw 12.5 µL of Taq Path Control Dilution Buffer from solution A and add to solution B. Vortex the mixture briefly and keep on ice prepared for this purpose.

3.2.3. Ethics

The study received approval from the Research Ethics Committee of the Doctoral School of Human and Veterinary Health Sciences at the University of Bangui (ESP/CE/78/2024). Participants provided verbal consent for data collection and analysis.

4. Results

4.1. Clinical Signs Observed in Patients with MPOX

Regarding clinical signs, 30% of patients' experience muscle pain followed by intense fever with lymphadenopathy (**Figure 1**).

4.2. Prevalence of Mpox Infections

Of 50 patients suspected of having Mpox sampled, 20% were confirmed positive for Mpox using RT-PCR.

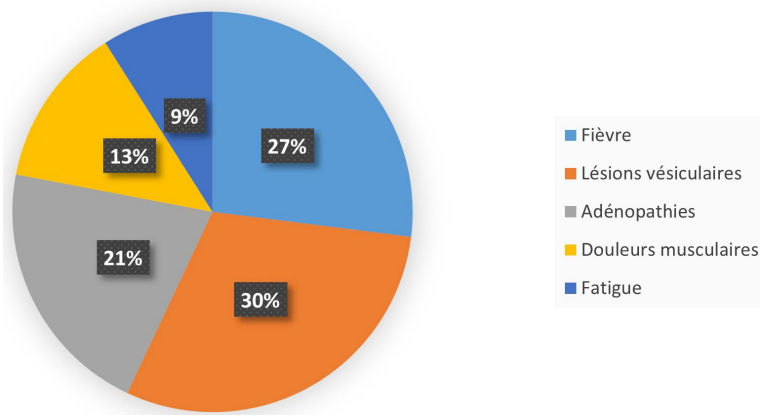

Figure 1. Symptoms observed in patients confirmed with Mpox.

4.2.1. Distribution of Mpox-Positive Patients by Age Group

According to the patients' condition, the most dominant age group is between 11 and 20 years, with a rate of 0.30% of Mpox-positive cases. The difference in Mpox-infected patients is not significant, with a $p \leq 0.70$ (Table 1).

Table 1. Distribution of Mpox-positive patients by age group.

Parametres	Results of RT-PCR				Total	p-value 0.05
	Positive	%	Negative	%		
Age in category (year)						
1 - 10 year	00	0.00	09	100.00	09	
11 - 20 year	03	0.30	07	70.00	10	
21 - 30 year	02	20.00	08	80.00	10	
31 - 40 year	01	14.29	06	85.71	07	0.70
41 - 50 year	02	33.33	04	66.67	06	
51 - 60 year	01	25.00	03	75.00	04	
>60 year	01	25.00	03	75.00	04	
Total	10	20.00	40	80.00	50	

4.2.2. Distribution of MPOX-Positive Patients by Gender

According to patient status, males are the most dominant gender, with a rate of 22.73% of MPOX-positive cases with a p-value of 0.46 (Table 2).

Table 2. Distribution of positive cases by gender.

Parametres	Results of RT-PCR				Total	p-value 0.05
	Positive	%	Negative	%		
Sex						
F	05	17.86	17	82.14	22	
M	05	22.73	23	77.27	28	0.46
Total	10	20.00	40	80.00	50	

4.2.3. Distribution of Positive Cases by Origin

Table 3 shows that MPOX infections in rural health districts are dominant, with a positivity rate of 21.05%. The difference is not significant, with a p-value of 0.57 (Table 3).

Table 3. Results of patients with RT-PCR by origin.

Parametres	Results of RT-PCR				Total	p-value 0.05
	Positive	%	Negative	%		
Origin						
Mbaïki	00	0.00	13	100.00	13	
Bagandou	06	33.33	12	66.67	18	0.07
Bangui	04	0.00	15	100.00	50	
Total	10	20.00	40	80.00	50	
Environment						
Rural	06	19.35	25	80.65	31	
Urban	04	21.05	15	78.95	19	0.57
Total	10	20.00	40	80.00	6278	

4.2.4 RT-PCR Curves

Figure 2 shows the different sigmoidal curves of DNA amplifications of Mpox-positive patients, and the linear curves are those of Mpox-negative patients (Figure 2).

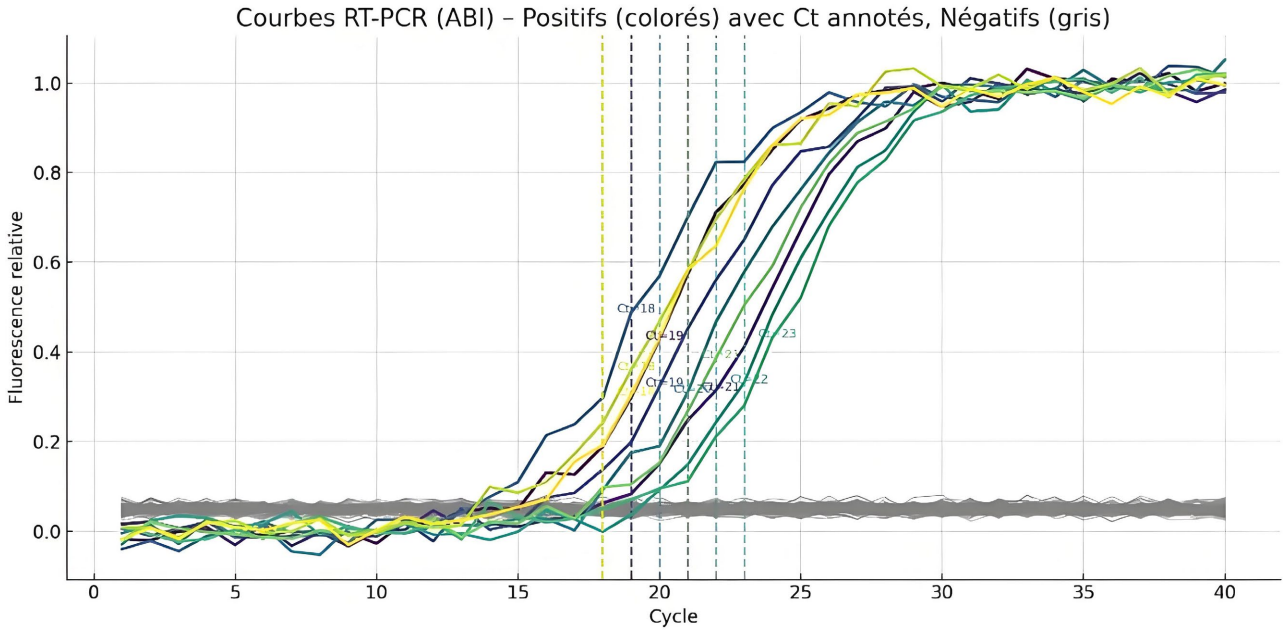

Figure 2. DNA amplification curves of Mpox-positive and -negative patients.

5. Discussion

Mpox surveillance in Africa in general, and in the CAR in particular, is hampered by a low case confirmation rate, although blood, scab, and lesion samples are rou-

tinely collected from all suspected patients. The reliability of these results was based on the quality of the data set methodologies used. This exclusivity allowed the target population to be the entire male and female population who participated in an Mpox investigation for diagnosis at the Bangui National Center for Public Health (LNBCSP).

The mean age of patients in this study was 29.20 years, ranging from 1 year to over 60 years (**Table 1**). A similar study conducted in Kinshasa in 2024 showed that the mean age of patients was 31 years [9]. This mean age found in this country is consistent with that found in this study. These could be explained by the fact that African populations in general and those of the CAR in particular are young. This able-bodied population is involved in several human-to-human activities and can easily become contaminated with this pandemic; this could also explain the influx of this age group to be tested. On the other hand, another study carried out by Bernard et al. in Europe in 2024 showed that the average age of patients was 21 years [9]. This difference in average age could be explained by the fact that the European population is aging, and that it is the younger ones who could agree to be tested and consent to such a study compared to the Central African population and African countries, which in the majority of cases quickly consent to diagnosis to detect the Mpox germ.

The median age was 25.50 years (**Table 1**). Another cumulative study conducted in Burundi and Uganda showed that the median age was 27 years [10]. The mean age obtained in this study is consistent with that obtained in Burundi and Uganda. This could be explained by the similarity of the populations in these different countries.

The results obtained were based primarily on polymerase chain reaction (PCR) diagnosis of suspected Mpox samples isolated from biological products such as blood, lesions, scabs, and oropharyngeal swabs (**Figure 2**). This prospective analytical study conducted at the LNBCSP recorded 50 samples: blood, lesions, scabs, and oropharyngeal swabs were isolated, representing a prevalence rate of 20% (**Figure 1**). This result is lower than that of a study conducted in the DRC in 2024 and 2025, in which suspected cases of isolated Mpox represented 8.8% of 44,078 samples. This difference could be explained by the fact that the DRC has several confirmed cases of Mpox per day and has had series of Mpox epidemics [11]. Suspected cases of Mpox were isolated at a rate of 5% in blood, 37% in lesions and 58% in scabs while a study conducted in Burundi shows that suspected cases of Mpox were isolated at a rate of 10% in blood, 27% in lesions and 58% in scabs [12].

This study confirmed cases of localized clade I Mpox with interhuman dissemination. These results are consistent with those of the DRC, Burundi, Uganda, and Congo Brazzaville, which also obtained cases of localized clade I Mpox with interhuman dissemination in their studies. But unlike the CAR, clade II circulates in these countries [13]. On the other hand, in CAR, a study carried out showed a male predominance of confirmed cases of clade I Mpox localized to human-to-

human dissemination among sex workers [14]. Studies carried out in the DRC, Burundi, Uganda, and Congo Brazzaville also showed the transmission of clade II Mpox among sex workers [15]. According to the WHO in 2025, these results are consistent with the results of a study carried out in Cameroon in 2024 and 2025 which noted a predominance of Mpox infections with human-to-human transmission.

Human transmission of clades I and II is favored by the APOBEC3 mutation, which is a cell-to-cell mutation [8]. It is the cause of rapid viral evolution worldwide and more particularly in the African context.

Using the RADI kit, real-time PCR for the diagnosis of Mpox aims at the target and gene: FAM (clade 2 for West Africa), HEX (clade 1 for Central Africa), Cy5 for the internal control, and Rox for the generic (**Figure 2**). These results highlight the need to strengthen the targeted vaccination policy in CAR and improve the early management of Mpox cases to limit transmission and reduce morbidity. Based on the above, vaccination is currently being introduced in CAR. This study has certain limitations such as the limited sample size (n = 50), resulting from convenience recruitment, which limits the statistical power and the representativeness of the results.

6. Conclusion

Monkey pox is responsible for serious infections with a high mortality rate. Analysis of MPXV cases diagnosed at the LNBCSP reveals active circulation of a clade I variant of MPXV in the CAR. The predominance of adaptive mutations and the observed clinical profiles call for strengthened surveillance, early diagnosis, and integrated management. The severity of the disease is even greater in the CAR due to the destruction of health facilities following the military-political crisis. Access to care for the population has become a real problem. Deforestation and hunting partly contribute to the emergence of this human infection. With monkey pox outbreaks now more frequent, districts neighboring infected areas must be closely monitored. Communication strategies and awareness campaigns must also be strengthened to avoid confusion and stigmatization.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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