Mpox outbreak in Rio de Janeiro, Brazil: a translational approach.

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# ABSTRACT

Mpox is a zoonotic disease historically reported in Africa. Since 2003, limited outbreaks have occurred outside Africa. In 2022, the global spread of cases with sustained interhuman transmission and unusual disease features raised public health concerns. We explore the mpox outbreak in Rio de Janeiro (RJ) state, Brazil, in an observational study of mpox-suspected cases from June to December 2022. Data collection relied on a public healthcare notification form. Diagnosis was determined by MPXV-PCR. In 46 confirmed cases, anti-VACV IgG was determined by ELISA, and seven MPXV genomes were sequenced. A total of 3,095 cases were included, 816 (26%) with positive MPXV-PCR results. Most positive cases were men in their 30s and MSM. A total of 285 (35%) MPXV-PCR+ patients lived with HIV. Eight were coinfected with varicella-zoster virus. Anogenital lesions and adenomegaly were associated with the diagnosis of mpox. Females and individuals under 18 represented 9% and 5% of all confirmed cases, respectively, showing higher PCR cycle threshold values and fewer anogenital lesions than adult men. Anti-VACV IgG was detected in 29/46 (63%) patients. All analyzed sequences belonged to clade IIb. In RJ state, mpox presented a diverse clinical picture, represented mainly by mild cases with low complication rates and prominent genital involvement. The incidence in females and children was higher than usually reported. The observation of a bimodal distribution of Ct values, with few positive results, may suggest the need to review the diagnostic criteria in these groups.

**Keywords:**

Monkeypox virus

Mpox outbreak

Sexual transmitted infection

Zoonotic disease

Females

Children

# INTRODUCTION

Mpox (formerly called monkeypox) is a zoonotic disease caused by the monkeypox virus (MPXV), a member of the Orthopoxvirus genus, which includes variola, vaccinia, and cowpox viruses1. The first documented human case was reported in an infant from the Democratic Republic of Congo in 19702,3. The disease has been endemic in Africa, leading to periodic outbreaks characterized by animal-to-human transmission with low secondary attack rates. Case identifications in Africa have revealed two clades: clade I (Central Africa/Congo Basin), with up to a 10% fatality rate, and clade II (West Africa), with approximately a 1% fatality rate4.

Literature reports suggest a gradual increase in mpox cases between 1980 and 2013, raising concerns about potential changes in the epidemiological pattern5,6. In 2003, 47 cases related to zoonotic transmission of MPXV from imported rodents occurred in the United States7. Subsequently, sporadic cases occurred in Israel (2018), the United Kingdom (2018-2022), Singapore (2019), and the United States (2021), involving travelers returning from endemic areas7,8. In May 2022, a cluster of mpox cases not associated with travelers was identified in the United Kingdom. This outbreak quickly spread to Europe, the Americas, the Eastern Mediterranean, and the Western Pacific regions, evolving into a public health emergency9.

The classical presentation of the disease is described as generalized skin eruptions, beginning on the face, preceded by systemic symptoms such as fever and lymphadenopathy. Incubation period can be as short as five days or as long as three weeks. Lesions progress through maculopapular, vesicular, pustular, and crust stages, each lasting 1-3 days and progressing simultaneously10. However, the 2022 outbreak has been characterized by atypical presentations with a few asynchronous lesions (simultaneous lesions at various stages), frequent involvement of the anogenital region, minimal systemic symptoms, and a concentration of cases among men who have sex with men (MSM) in a sustained chain of interhuman transmission11,12.

Mpox has several differential diagnoses among acute skin eruption diseases, mainly those of infectious etiologies13,14, including varicella, herpes simplex, syphilis, gonorrhea, and vaccinia15. The presumptive mpox diagnosis based solely on clinical features demonstrates low accuracy16, underscoring the importance of laboratory confirmation. The gold standard methods for diagnosing mpox are viral isolation, electron microscopy, and real-time PCR5, the latter being extensively used during the 2022 outbreak. Preliminary CDC case definitions rely on these methods to determine probable and confirmed cases, including the detection of anti-orthopoxvirus IgM antibodies up to 56 days after the onset of skin eruptions17. However, serological methods were not commonly employed for diagnosis during this outbreak.

The first reported case of mpox in Brazil occurred on June 9th, 2022, in São Paulo18. Until July 2023, 10,950 confirmed cases and 16 deaths had been reported19. Rio de Janeiro (RJ) represented the second state in absolute number of confirmed cases and the first in deaths. The Universidade Federal do Rio de Janeiro (UFRJ), through its Núcleo de Enfrentamento e Estudo de Doenças Infecciosas Emergentes e Reemergentes (NEEDIER), played a significant role in the public health response in Brazil. NEEDIER-UFRJ received samples to confirm the mpox diagnosis from eight states, including RJ, and provided on-site support to patients suspected of mpox. This study presents clinical, epidemiological, serological, and molecular findings from mpox-suspected patients from RJ diagnosed at our reference center in Brazil, expanding the understanding of the MPXV infection and contributing to delineating a rational strategy for disease control.

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# 2. MATERIALS AND METHODS

## 2.1. Study design and population

We conducted an observational study including suspected cases of mpox from the RJ state, tested at UFRJ between June 4th, 2022, and December 31st, 2022, with available clinical data. A subset of patients received on-site evaluation at NEEDIER. Probable cases included individuals of any age who, after March 15th, 2022, presented single or multiple acute skin lesions suggestive of mpox (deep and well-defined lesions, usually with central umbilication, at maculopapular, vesicular, pustular, or crust stages) in any body region, with or without fever or adenomegaly20.

## 2.2. Data collection and laboratory procedures

Age, sex, gender, clinical presentation, sexual exposure, travel history, HIV status, non-HIV immunosuppression, and the simultaneous presence of sexually transmitted infection other than HIV (STI) of suspected mpox cases were collected using a public healthcare notification form (the REDCap® electronic data). Additional clinical and epidemiological information was available for a subgroup of patients who were assessed at our reference center. In this subgroup, confirmed mpox cases were monitored weekly and discharged based on clinical criteria.

Mpox diagnosis was established by analyzing swabs from skin lesions (content or fragments), oropharynx, and/or anal region. A triplex real-time PCR assay was employed to detect monkeypox virus DNA (7500 Thermal Cycler, Applied Biosystems, USA), targeting the F4L gene for orthopoxvirus (OPXV), the G2R for both MPXV clade I and II, and the human RNase P (RP) as an endogenous control. Alternatively, a 4-plex real-time PCR assay that also targets varicella-zoster virus (VZV) (Kit Molecular 4Plex OPXV/MPXV/VZV/RP, Bio-Manguinhos/Fiocruz, 2022) was utilized following the manufacturer's instructions21. Samples were considered positive when PCR cycle threshold (Ct) values were below or equal to 37 and inconclusive between 37 and 4022.

In the subset of patients who were attended at our reference center, anti-VZV IgM and IgG (Euroimmun, Germany) and anti-VACV IgG (vaccinia virus Wyeth clone A111) titers were determined by semi-quantitative ELISA from serum23. Additionally, MPXV isolation in cell culture was performed from swab samples as previously described24. MPXV isolate was fixed in 2.5% glutaraldehyde and analyzed by scanning electron microscopy (SEM)25. For MPXV genome sequencing, samples were selected (Ct<28) and PCR amplified using the COVIDSeq kit (Illumina, USA), with modifications as previously described26. Pooled libraries were sequenced using 2x100 bp pair-end Illumina flow cell kits for NextSeq2000. The raw data were base-called and trimmed using Illumina’s Basespace data cloud. Reads were mapped to a reference genome (NC\_063383.1) using the Geneious Prime software. Consensus sequences were generated with a minimum coverage of 10x. For clade assignment, mutation calling, and quality checks, consensus sequences were submitted to Nextclade (accessed on September 11th, 2023) using the hMPXV dataset.

This study received approval from the Research Ethics Committee of the HU-UFRJ (Reg\_62281722.5.0000.5257). Participants who were assessed on-site provided written informed consent for study participation and additional consent for their anonymized images use. Informed consent of external participants was waived because of the deidentified nature of the database.

## 2.3. Statistical analysis

We provided descriptive statistics for demographic variables. We used logistic regression to estimate the probability of mpox diagnosis based on the independent symptoms in the whole cohort and individual odds ratios (ORs) among females, excluding patients with an inconclusive MPXV-PCR test. We performed Silverman’s bandwidth test to evaluate multimodality of MPXV-PCR Ct values. In the subset of patients with serum samples, we calculated a Kendall correlation between their lowest MPXV-PCR Ct values and their highest anti-VACV IgG titers. We used the Wilcoxon rank-sum test to compare Ct values in subsets of patients. A *p*-value < 0.05 was considered significant. Statistical analyses and data visualization were performed using R software version 4.3.0.

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# 3. RESULTS

## 3.1. Spatial, temporal, and demographic description

A total of 3,571 individuals with suspected mpox were tested, resulting in 931 (26.0%) confirmed cases through MPXV-PCR. The majority of suspected and confirmed mpox cases were concentrated in the city of Rio de Janeiro, including the tourist district of Copacabana and city center neighborhoods (Figures 1A-B). The rolling average of cases peaked in August, decreased during September, with only a few remaining individuals diagnosed in November and December (Figure 1C).

Among the 3,571 suspected mpox cases from RJ, 3,095 had available clinical information for further analysis (Table 1). Most of them were male (1,965; 63.5%) with a median age of 30 years (IQR 19-42). Five hundred eighty-one (52.0%) men reported having sex with men, and 369 (26.1%) reported having multiple or unknown sexual partners. Three hundred and seventy suspected cases (12.0%) reported living with HIV, 26 (7.0%) of them with CD4 count under 200 cells/mm³. Other active STIs affected 105 patients (3.5%), syphilis being the most common.

Females represented 1,130 (36.5%) of all suspected cases, with a median age of 29 years (IQR 17-45). Only 28 females reported sex with multiple or unknown partners (2.5%). Individuals under 18 years accounted for 675 (21.8%) of the cases, with a median age of 10 (IQR 5-14); 57% were males.

Among the 3,095 suspected cases from RJ with clinical data, 816 (26.4%) had a positive PCR result (confirmed cases), including 44 (5.3%) individuals under 18 years. Females comprised 9.4% of confirmed cases (77/816) and 38.6% of individuals under 18 years (17/44). Of the 77 females with confirmed mpox, all but one were cis women. Two MPXV-PCR+ females were pregnant, one in the first and one in the second trimester. A total of 119 patients had inconclusive MPXV-PCR results (3.8%).

Of the 816 confirmed cases, 702 (86.0%) had no information about the probable source of infection. Five patients, diagnosed in the first weeks of the RJ outbreak, reported international travel within the three weeks preceding symptom onset, primarily from Spain, Europe's epicenter27. Table 1 displays the individual characteristics, behavioral traits, and STI diagnoses in patients confirmed and non-confirmed for mpox.

The subset of patients seen at NEEDIER (n = 193) were predominantly male (128; 66.3%) with a median age of 31 years (IQR 21-40). In this group, 68 patients had MPXV-PCR+ (35.2%), a positivity rate slightly higher than that observed for the entire RJ state.

## 3.2. Clinical presentation of RJ confirmed cases

The median time from the onset of symptoms to the first sample collection was five days (IQR 3-8) in confirmed cases. Most cases were mild to moderate and managed on an outpatient basis. Thirty-seven confirmed cases required hospitalization, six in intensive care: 30 for pain and secondary infection management, and 7 for isolation (Table 1). Four deaths occurred in our cohort  (three males and one female), all of them had comorbidities: two men had HIV and CD4 counts < 50 cells/mm³ (33 and 31 years old), one man had tuberculosis and was on corticosteroids (27 years old), and one bedridden woman (69 years old), who had been on dialysis since 2022 due to chronic kidney disease, had diabetes, hypertension, and uterine cervix neoplasm with a vaginal fistula.

Six hundred and ninety-five (85.2%) individuals with confirmed diagnosis presented skin eruptions. Systemic symptoms were also prevalent, including fever (473; 58.0%), lymphadenopathy (370; 45.3%), headache (347; 42.5%), asthenia (261; 32.0%), and myalgia (254; 31.1%). The symptoms most strongly associated with the mpox diagnosis were anogenital lesions (OR = 2.79; 95% CI 2.26-3.45), lymphadenopathy (OR = 2.72; 95% CI 2.19-3.38), and fever (OR = 1.95; 95% CI 1.57-2.43) (Figure 2A).

Among female patients (n = 77), skin eruption was also the most frequently reported symptom (65; 84.4%), followed by fever (36; 46.8%), headache (33; 42.9%), myalgia (22; 28.6%), and asthenia (19; 24.7%). In this group, almost half of the patients had lesions on the upper or lower limbs (42.8% and 46.7%, respectively) and trunk (37.6%), followed by anogenital lesions (24.6%). Anogenital lesions were the sign most strongly suggestive of mpox diagnosis (OR 2.18; 95% CI 1.24-3.69) (Figure 2B). Young patients, under 18 years (n = 44), presented a similar clinical picture, with skin eruption (39; 88.6%), fever (23; 52.2%), headache (14; 31.8%), sore throat (11; 25%), and myalgia (9; 20.4%) as the main symptoms. Lesions were also more frequent on the upper and lower limbs (54.5% each), trunk (50.0%), and face (47.7%). Anogenital lesions were observed in 9 subjects (20.4%), five between 14 and 17 years old.

Among patients with inconclusive MPXV-PCR results (n = 119), the most reported symptom was skin eruption (102; 85.7%), followed by fever (36; 30.2%), headache (32; 26.8%), and lymphadenopathy (22; 18.4%). Most patients with inconclusive MPXV-PCR results were male (65; 54.6%). As in female patients, the anogenital region was only the fourth most common location of lesions in patients with inconclusive results (23.5%), after the upper limbs (42.8%), trunk (36.9%), and lower limbs (35.2%) (data not shown).

In the subset of confirmed patients who were seen in our reference center (68/193), we could discriminate and analyze lesion morphology in detail (Figures 3A-F). Most patients (59/68; 86.7%) had multiple lesions, and only 20.5% (14/68) showed lesions at the same stage of development. Skin eruptions occurred before other symptoms in 22 patients (32.3%). The lesions were painful and pruritic in about half of the cases (44.1% and 51.4%, respectively). Crusts were the most common lesion type, present in 38 patients (55.8%), followed by pustules (30; 44.1%) and ulcers (27; 40%). Among females (10/68) and young patients (7/68), lesions were less characteristic regarding location but were mainly crusts. Both groups reported pruritic lesions, painful in females and painless in young patients.

## 3.3. Laboratory results

## In RJ state, the median Ct value of MPXV-PCR tests for confirmed patients was 19.2 (IQR 16.8-23.8). Females exhibited significantly higher and more dispersed Ct values (34.4; IQR 23.0-35.9) than males (18.8; IQR 16.6-22.1), showing a bimodal distribution (Figures 4A-B).

When considering a positive cutoff value of 34 instead of 3728, the median Ct values for females were still higher than for males, although a reduced difference and dispersion were observed (Figures 4C-D). Patients under 18 years had Ct value characteristics similar to those observed among females (Figures 3E-F).

We also compared Ct values distribution among lesion types and anatomical sites: vesicles displayed the lowest Ct values, followed by umbilicated lesions and pustules (Figure 5A). Skin samples collected from the scalp, perineum, and penis had the lowest Ct values, followed by those from the face or neck, and beard or mustache (Figure 5B). In our experience, darkened crusts surrounded by slightly raised, purulent rings in regions of facial hair strongly suggested a diagnosis of mpox (“*pastel de nata*" lesions) (Figure 3B).

As an important differential diagnosis for mpox, varicella was investigated by PCR and/or serology. One hundred and eighty-nine patients received a diagnosis of varicella: 178 by PCR (Ct value < 37) and 12 by serology (IgM titer > 1.5), with one patient diagnosed by both VZV-PCR+ and IgM+. Among patients with confirmed varicella, eight also had confirmed mpox, indicating active co-infections (four VZV-PCR+ and five IgM+, one patient had both VZV-PCR+ and IgM+). Our first case of co-infection was diagnosed in mid-July 2022, a month earlier than similar cases currently reported as the first in the global outbreak29. Eight patients with confirmed VZV (one by IgM and seven by PCR) had an inconclusive MPXV-PCR result, while nine patients with confirmed mpox had an inconclusive diagnosis of VZV (two by PCR and seven by IgM).

Forty-six patients with confirmed mpox who attended NEEDIER provided serum samples for anti-VACV IgG analysis. Seventeen patients (36.9%) were seropositive at the first appointment and maintained detectable IgG titers throughout follow-up. Twelve patients seroconverted during follow-up (26.1%), five showed IgG+ within the first seven days of the disease. Seventeen patients had no detectable IgG during follow-up (Figure 5C). Only one female seroconverted and no patients under 18 years showed seroconversion, correlating with low viral load as inferred from Ct values (R = -0.49; p < 0.01) (Figures 5D-E).

We isolated MPXV in cell cultures from swab samples collected from 7 patients who attended NEEDIER. SEM of isolates demonstrated virus-like particles compatible with MPXV (≃ 225 nm size and brick-shaped morphology, Figure 6). We sequenced seven MPXV genomes (10%). The seven genomes had good overall quality according to Nextclade (https://clades.nextstrain.org/), and all belonged to clade IIb, including lineages B.1 (n = 3), B.1.9 (n = 3), and B.1.1 (n = 1). The genome sequences are publicly available on GISAID (EPI-numbers 18241786 to 18241792).

# 4. DISCUSSION

We diagnosed 816 cases of mpox among 3,095 individuals enrolled in our Rio de Janeiro cohort from June to December 2022, peaking in August. The cases occurred predominantly among men, mostly men who have sex with men (MSM), in their third and fourth decades of life. The number of mpox cases among females and individuals under 18 years was higher in our cohort than typically reported (9.4% and 5%, respectively). We observed a diverse clinical picture, ranging from asymptomatic cases or isolated skin lesions to generalized skin eruptions with systemic symptoms and infrequent complications. Skin lesions were the most frequent symptom, while anogenital lesions were the sign most strongly suggestive of mpox diagnosis in adults. We also documented seroconversion in a subgroup of patients, and co-infection of mpox and varicella, both findings less commonly reported in the 2022 outbreak.

Our findings align with those documented in the 2022 mpox outbreak in other countries and corroborate observations such as frequent uncertainty about the infection source11,12,30,31 and a small proportion of confirmed cases with international travel history30,32. This suggests that a few cases are sufficient to establish regional and local transmission and highlights the need to maintain global surveillance.

In accordance with other studies12,33, no substantial clinical differences were found between people living with HIV and HIV-seronegative patients, except for more disseminated and exuberant lesions, as well as more frequent complications and hospitalization among patients with low CD4 counts. In fact, some authors have proposed severe mpox as an AIDS-defining condition34,35. Several studies on this outbreak have been designed around sexual health facilities or HIV clinics33,36,37, allowing the  focus on  patient sexual history and concurrent STI diagnosis. Many reports show a prevalence of STIs in up to a third of  mpox confirmed cases12,32,33. We reported a low rate of coinfection with STIs other than HIV, which can be explained by limited screening and underdiagnosis.

Cisgender women are infrequently reported as cases of mpox in the 2022 outbreak. In many cohorts and case series, no patients were identified as women. In the few studies that did describe infections in women, they represented less than 5% of the study population32,33,38,39. Of note, studies focusing on females show a common misdiagnosis and delayed diagnosis in cis women40, suggesting a slightly different clinical picture41. Our study, however, found that females represented 9% of all confirmed cases, allowing for further characterization of clinical findings in this group. Females had less lymphadenopathy and anogenital lesions, with more lesions on the extremities and notably less sexual exposure reported than males.

Similarly, individuals under 18 years were overlooked in the 2022 outbreak. However, the first human mpox case ever reported was a 9-month-old infant2,3, and minors are frequently infected with monkeypox virus in endemic countries5,42–44. Additionally, minors represented a third of confirmed cases during the first outbreak of mpox outside Africa in 200345. Few studies from the 2022 outbreak specified patients under 18 years32,41, and some actively excluded them from analyses. A CDC case series from the 2022 outbreak outlines minors representing less than 0.5% of total cases in the United States46, which can be an effect of structuring study designs around sexual health facilities rather than broader surveillance scenarios.

Detailing the findings in the young population is important considering numerous exanthematous diseases primarily affecting infants and minors, such as varicella, molluscum contagiosum, and hand-foot-and-mouth disease. Lesion characteristics could improve the distinction between mpox and other disorders characterized by skin lesions14. However, an overlapping spectrum of these diseases precludes certainty in differential diagnosis on a clinical basis alone. Co-infection of monkeypox virus and another infectious agent is also a possibility. Indeed, cases of co-infection of MPXV and VZV have been documented in mpox-endemic countries42,47–49 and recently in an adolescent girl during the 2022 mpox outbreak29. Our findings expand observations of MPXV and VZV co-infection outside Africa.

In addition to clinical and epidemiological perspectives, this work also sheds light on molecular diagnostic findings among females and minors. Samples from these groups displayed significantly higher and more dispersed Ct values in MPXV-PCR, which inversely correlates with low viral load. However, reducing cutoff values for positivity brings median Ct values obtained for females and minors closer to those observed in adult men. This strategy could reduce the chances of false positive results and has been used previously46. We underscore the need to carefully interpret these results in low pretest probability settings and suggest revising the standards for laboratory diagnosis in these groups. Our data support and expand previous findings on the mpox outbreak in RJ32, suggesting that although sexual contact shows a relevant role in mpox transmission dynamics, the relatively high number of observed cases among females and minors undelies the need to consider the diagnosis in other populations as well.

The cross-immunity among orthopoxvirus species allows the use of vaccinia virus (VACV) strains as antigen in ELISA tests for mpox investigation23.  Previous work reported anti-VACV IgM and IgG determination to  support a retrospective differential diagnosis between mpox and varicella within a family cluster50. In this context, our cohort provides relevant information on seroconversion dynamics after mpox infection. The early detection of anti-VACV IgG in our cohort could be explained by previous smallpox vaccination and imprecise symptom reporting in oligosymptomatic individuals.

Our findings support that females and minors with mpox develop a less pronounced disease form, without a clear epidemiologic link and likely acquired as in other contact diseases such as varicella (without sexual exposure). Diverse clinical presentations and higher MPXV-PCR Ct values could be associated with a lower viral load inoculum than observed in patients with sexual exposure. Accordingly, serological evaluation of anti-VACV IgG showed reduced sensitivity in mild disease. Indeed, we detected anti-VACV IgG mainly in males with classical mpox lesions and clear epidemiologic links, while females and minors had undetectable anti-VACV IgG.

Our study has some limitations. First, clinical data collected using the REDCap® tool is restricted to pre-structured questions, which may hinder detailed lesion characterization and specific analyses. Second, some health-care institutions do not notify cases systematically, preventing broader analysis of all tested cases. Third, patient samples were eventually pooled, potentially affecting final Ct values. Lastly, serum samples were only available for patients attended at our reference center, which may limit the generalizability of our findings regarding anti-VACV IgG results.

Our cohort represents an extensive single-center case series, yielding broadly informative and detailed analysis of confirmed patients. We reported a significant number of cases in females and children that, aligned with a multidisciplinary approach, allowed the identification of specific virological and serological dynamics, expanding the MPVX infection understanding.

As documented in the current outbreak, there is high clinical diversity in how mpox may manifest, reinforcing the importance of laboratory confirmation. Globally, healthcare professionals must become familiar with the diverse features of mpox and be aware that, despite MSM having been disproportionately affected in the current outbreak, the disease may spread to other populations, as our results have highlighted for females and children. Mpox is not gone, and there are still knowledge gaps. The disease persists and expands in Africa. In parallel, regional and international movement of people increases exponentially. Consequently, there are no borders for MPXV dissemination and non-endemic outbreaks may recur, potentially spreading more virulent strains.

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AUTHORS CONTRIBUTION

Conceptualization: GSL, VAO, MQSM, RMG, DSF, AT, TMPPC. Methodology: GSL, VAO, DM, OCFJ, CRD, RMG, DSF, AT, TMPPC. Data collection: GSL, VAO, MQSM, ACPC, ICL, MH, TA, LAVS, DGMR, ACC, SC. Data curation: GSL, VAO, CMV, OCFJ, CRD, RMG, DSF, TMPPC. Performance of PCR tests: DM, CCAG. Performance of serological tests: NVCC, LJR, OCFJ, CRD. Scanning electron microscopy: FAC, LAC, WS. Genomic sequencing: LPOL, VLV, CMV, Formal analysis: GSL, VAO, RMG, DSF. Validation: OCFJ, CRD, RMG, DSF, AT, TMPPC. Visualization: GSL, VAO, MQSM, ACPC, RMG, DSF, TMPPC. Supervision: ACPC, BOS, RMG, TMPPC, OCFJ. Project administration: AT, TMPPC. Funding acquisition: CRD, AT, TMPPC. Writing-original draft: GSL, VAO, MQSM, ICL, DSF, TMPPC. Writing-reviewing and editing: GSL, VAO, MQSM, ACPC, BOS, LMH, CRD, RMG, DSF, AT, TMPPC. All authors reviewed and approved the submitted manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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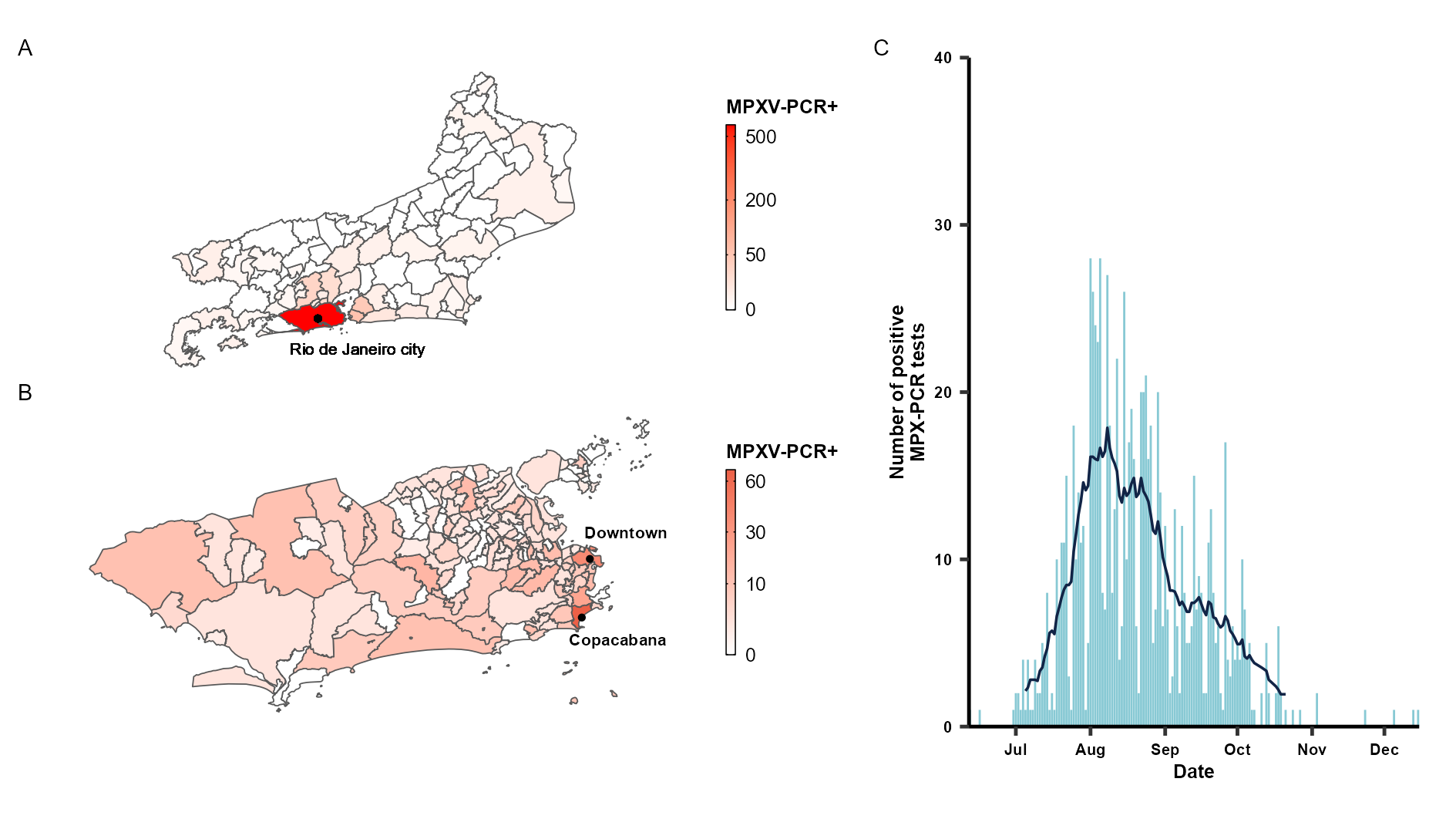
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# **7. FIGURES AND DATA**

## Figure 1

**Figure 1.** Demographic and temporal distribution of mpox-confirmed cases by MPXV-PCR in Rio de Janeiro (RJ), Brazil, diagnosed at NEEDIER from June 04th, 2022, to December 31st, 2022. **A**, mpox-confirmed cases (log scale) in RJ State, showing the highest incidence in Rio de Janeiro City. **B**, mpox-confirmed cases (log scale) within the city of Rio de Janeiro. **C**, number of positive MPXV-PCR in RJ State from June to December 2022, daily (blue bars) and seven-day rolling average (black line).



## 

## Table 1

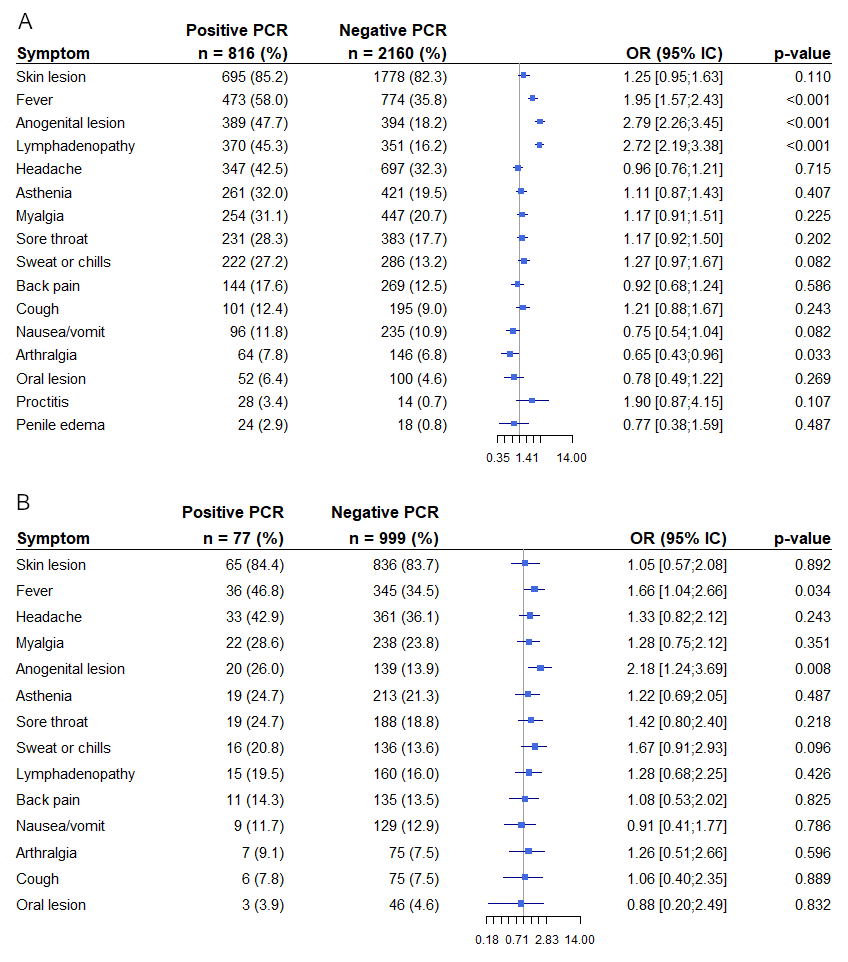
**Table 1.** Clinical and epidemiological characteristics of mpox-suspected patients in Rio de Janeiro state from June to December, 2022.

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **MPXV-PCR\*** | |
|  | **Suspected cases**  **n (%)** | **Positive**  **n (%)** | **Negative**  **n (%)** |
| **Total** | 3095 | 816 | 2160 |
| **Sex** |  |  |  |
| Male | 1965 (63.5) | 739 (90.6) | 1161 (53.8) |
| Female | 1130 (36.5) | 77 (9.4) | 999 (46.3) |
| **Age** |  |  |  |
| Under 18 | 675 (22.0) | 44 (5.4) | 607 (28.1) |
| 18 to 39 | 1505 (48.6) | 558 (68.4) | 897 (41.5) |
| ≥ 40 | 915 (29.6) | 214 (26.2) | 656 (30.4) |
| **Skin colour** |  |  |  |
| White | 1127 (36.4) | 324 (39.7) | 761 (35.2) |
| Non-white | 1464 (47.3) | 414 (50.7) | 987 (45.7) |
| **MSM\*\*** | 581 (52.0) | 430 (85.0) | 137 (24.1) |
| **Intimate contact with unknown and/or multiple partners\*\*** | 369 (26.1) | 244 (48.6) | 112 (13.1) |
| **Intimate contact with suspected or confirmed mpox case\*\*** | 280 (12.9) | 115 (19.2) | 151 (10.2) |
| **International travel in the last 21 days** | 10 (0.3) | 5 (0.6) | 5 (0.2) |
| **PLWH** | 370 (12.0) | 285 (34.9) | 74 (3.4) |
| CD4 count < 200 | 26 (7.0) | 20 (7.0) | 6 (8.1) |
| CD4 count ≥ 200 | 231 (62.4) | 183 (64.2) | 45 (60.8) |
| Unknown | 113 (30.5) | 82 (28.8) | 23 (31.1) |
| **Active STI other than HIV** |  |  |  |
| Syphilis | 92 (3.0) | 51 (6.3) | 39 (1.8) |
| Gonorrhea | 5 (0.2) | 4 (0.5) | 1 (0.05) |
| Other | 8 (0.3) | 1 (0.1) | 6 (0.3) |
| **Varicella zoster infection** | 189 (6.1) | 8 (1.0) | 173 (8.0) |
| **Asymptomatic** | 103 (3.3) | 9 (1.1) | 91 (4.2) |
| **Hospitalization** | 123 (4.0) | 37 (4.5) | 81 (3.8) |
| **ICU admission** | 19 (0.6) | 6 (0.7) | 12 (0.6) |
| **Death** | 8 (0.3) | 4 (0.5) | 4 (0.2) |
| **Past smallpox vaccine** | 107 (3.5) | 23 (2.8) | 75 (3.5) |
| **DSSO [median (IQR)]** | 5 [3-8] | 5 [3-8] | 5 [2-9] |

\*Inconclusive MPXV-PCR results = 119. \*\*Percentages calculated for nonmissing answers (excluding females and minors for MSM). MSM: men who have sex with men. PLWH: person living with HIV; STI: sexually transmitted infection; DSSO: days since symptom onset.

## Figure 2

**Figure 2.** Forest plots showing symptom frequencies and multivariable logistic regression (**A**) or univariable (**B**) odds ratios with 95% confidence intervals and p-values of factors associated with mpox diagnosis in all (n = 2976, **A**) and female (n = 1076, **B**) mpox-suspected cases according to MPXV-PCR result.



## Figure 3

**Figure 3.** Cutaneous manifestations of mpox in patients attended at NEEDIER. (A) Pleomorphic lesions on the forehead of an 18-year-old male with mpox and varicella co-infection. (B) Perioral "*pastel de nata*" lesion in a 38-year-old male. (C) Multiple umbilicated penile lesions with surrounding whitish papules in a 59-year-old male. (D) Penile lesions on a smallpox-vaccinated 46-year-old male; lesions registered four days after symptom onset (D.1) coalesced into an ulcer with erythematous halo fifteen days later (D.2). (E) Perianal umbilicated vesicles in a 45-year-old male. (F) Two perianal ulcers (F.1) and a punctiform, erythematous, crusted papule on the forearm (F.2) in a 24-year-old female.

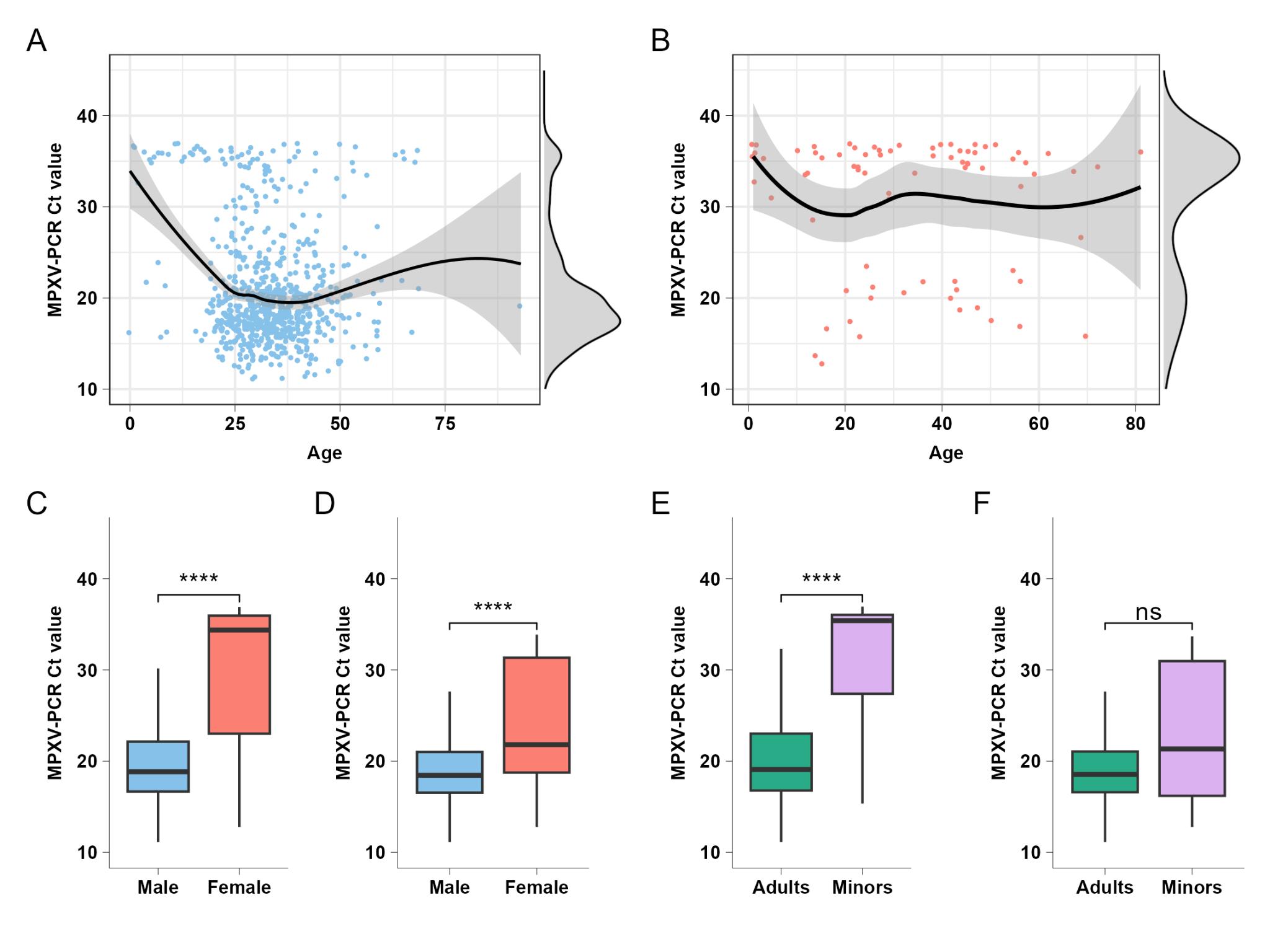
**Foto de homem fazendo careta

Descrição gerada automaticamente com confiança baixa**

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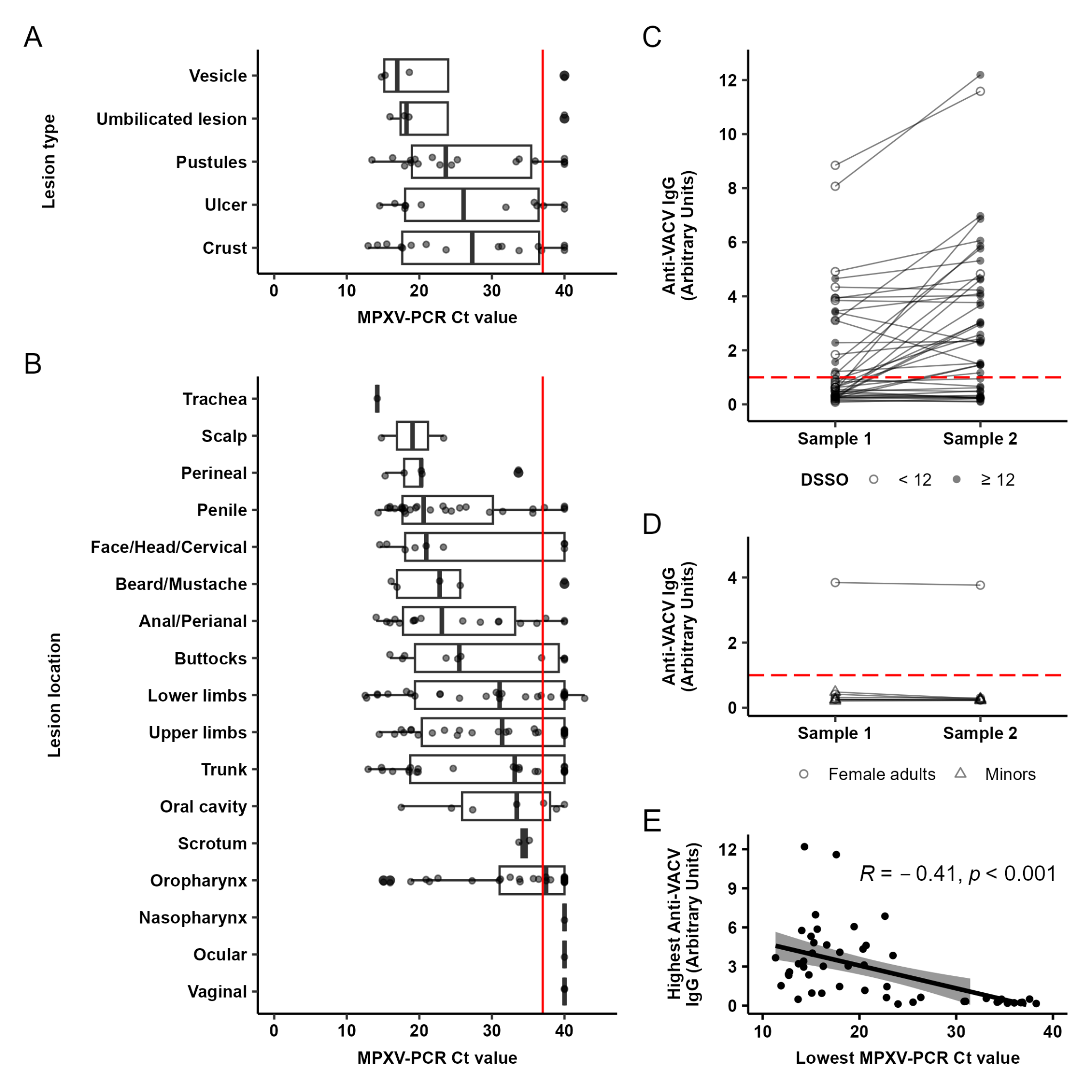
## Figure 4

**Figure 4.** MPXV-PCR cycle threshold (Ct) values in mpox-confirmed cases. Scatter plots show Ct value distribution according to age, in males (**A**,n = 735) and females (**B**,n = 61), with local smoothing (solid line) and density plots (right y-axis). Lower panels show MPXV-PCR Ct values in male *vs.* female mpox-confirmed cases (**C**, **D**) and in individuals over (adults, n = 761) *vs.* under 18 years old (n = 35) (**E**, **F**), considering standard cutoff values of 37 (**C** and **E**) and modified cutoff value of 34 (**D** and **F**). \*p-value < 0.0001, ns = non-significant.



## Figure 5

**Figure 5.** MPXV-PCR Ct values distribution according to lesion type (**A**) and location (**B**) in mpox-confirmed cases who were attended at NEEDIER (n = 63). Vertical red line shows the cutoff value (37). Semiquantitative anti-VACV IgG titers determined in the first and second medical appointments of all 46 patients who were attended at NEEDIER. The higher titers were obtained in previous smallpox vaccinated individuals (**C**) and for females and minors (**D**). DSSO, days since symptom onset. **E**, Kendall correlation plot between the lowest MPXV-PCR Ct value and the highest anti-VACV IgG titer obtained in 31 patients who were attended at NEEDIER.



## Figure 6

**Figure 6.** Electron microscopy of MPXV isolate. (A) Scanning electron microscopy of samples collected from pustules of a MPXV-infected individual revealed virus-like particles consistent with size and morphology of cell-associated enveloped virus (CEV) on the cell surface (arrows). (B) Panoramic view of BSC40 cells infected with MPXV isolate. Clusters of mature viruses (MV) were highlighted by arrows. (n) nucleus; (m) mitochondria.

