

Epidemiological report

## Epidemiological report: first confirmed cases of monkeypox

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## INTRODUCTION

Monkeypox is a zoonotic infection caused by the monkeypox virus, which mostly occurs in West and Central Africa. Despite of the name, those animals do not participate in human transmission. All cases which were hitherto identified by global health agencies were ascribed as human-to-human transmitted. In Portuguese, the use of the name in English, “monkeypox” (MPX), is recommended, as to avoid stigmatization and actions against non-human primates (NHP).<sup>1</sup>

The virus is mainly transmitted through direct or indirect contact with blood, body fluids, skin rashes, or mucous membranes of infected animals. Human-to-human transmission mainly occurs through close contact with skin rashes of infected people, or through respiratory tract secretions, as well as secretions in objects, cloth, and surfaces used by the diseased person. Viral transmission via respiratory droplets usually requires closer contact between the infected patient and other people, putting health professionals, family members, and other contacts at a higher risk of infection.<sup>2-4</sup>

The incubation period is typically 6 to 13 days, and the interval time can range from 5 to 21 days. After being infected, the person initially shows symptoms like fever, myalgia, fatigue, headache, asthenia, back pain and lymphadenopathy. After three days, 1-3 of prodrome, the individual shows centrifugal maculopapular eruptions, starting on the site of the primary infection, and spreading rapidly to other body parts. Usually, within 12 days the lesions progress from the macula stage to papules, vesicles, pustules, and crusts. The difference in aspect from chickenpox or syphilis is the more uniform evolving of rashes. When they crust over, the person no longer infects other individuals, which occurs on average within 2-4 weeks.<sup>4,5</sup>

The occurrence of monkeypox in non-endemic countries is associated with traveling to places where the disease is endemic, transmission from imported animals, or contact with documented epidemiological association with imported cases.<sup>4,6</sup>

Since 1958, when monkeypox was initially described, many non-endemic countries have reported outbreaks. However, the 2022 outbreak presents the highest number of confirmed cases, as well as the wider global distribution.

Up to June 29, 2022, 5,179 suspected cases were reported; 5,135 cases were confirmed in many countries: United Kingdom (1,076), Germany (875), Spain (800), France (440), Portugal (391), United States (351), Canada (276), Netherlands (257), Italy (159),

Belgium (117), Switzerland (81), Israel (38), Ireland (28), Brazil (21), Austria (20), Ghana (18), Denmark (18), Norway (17), Sweden (13), Australia (13), United Arab Emirates (13), Hungary (12), Poland (12), Mexico (11), Romania (11), Slovenia (9), Czech Republic (6), Chile (6), Finland (4), Malta (4), Argentina (4), Greece (3), Iceland (3), Luxembourg (3), Benin (3), Peru (3), Colombia (3), Bulgaria (3), Latvia (2), South Africa (2), Gibraltar (1), Morocco (1), Venezuela (1), Georgia (1), Serbia (1), Lebanon (1), South Korea (1), Singapore (1), Taiwan (1), and Croatia (1).<sup>6</sup>

In the same period, Brazil reported 99 cases, 21 of which were confirmed: 14 in São Paulo; 5 in Rio de Janeiro; and 2 in Rio Grande do Sul. As established by the Brazilian Department of Health, samples from suspected MPX cases must be processed at one of the four reference laboratories for the disease in the country: Adolfo Lutz Institute (IAL), serving the states of São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul; Ezequiel Dias Foundation, serving Minas Gerais and all the North Region; the Enterovirus Lab at Oswaldo Cruz Foundation, serving Rio de Janeiro and the Northeast Region; and the Viral Molecular Biology Lab at the Biology Institute, in the Federal University of Rio de Janeiro (UFRJ), serving Espírito Santo and the states in the Central-West Region.<sup>5</sup>

At IAL, the Strategic Lab (LEIAL) is responsible for processing clinical samples, collecting lesions, detecting monkeypox virus through real-time PCR, and sequencing positive cases. LEIAL has confirmed all reported cases in the states of São Paulo and Rio Grande do Sul.

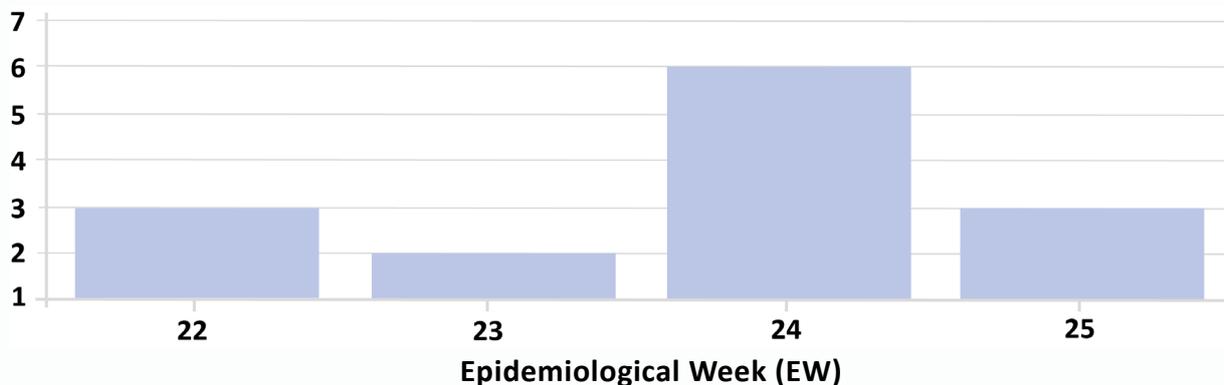
## METHODS

We have obtained clinical and epidemiological data concerning the current monkeypox outbreak from notifications in the CeVeSP System (<https://cevesp.saude.sp.gov.br>), a dynamic web platform for reporting events and aggravations of public health interest. The IT team from the Strategic Information Center for Health Surveillance in the State of São Paulo (Central CIEVS/SP) has developed the system in order to enable quick case reporting and epidemiological research. The CeVeSP System also issues real-time reports, assesses on-call production, provides means for identifying and following up events, monitors contacts, and helps investigating emergency aggravations requiring quick, effective response.

For real-time PCR, we have extracted viral DNA from clinical material using the MPTA-PU16 kit for pathogen DNA and RNA extraction (Loccus, São Paulo, Brazil), in the Extracta-32 equipment (Loccus, São Paulo, Brazil). The extracted DNA was tested according to the protocol of Li et al. (2010), in order to detect the presence of monkeypox virus genetic material.<sup>7</sup>

The real-time PCR positive cases were sequenced. To quantify the materials, we have used the Quibit dsDNA HS Assay kit (ThermoFisher Scientific, Waltham, MA, USA) accordingly to the manufacturer’s protocol. From the total DNA, we have assembled libraries compatible with the Illumina platform (NEXTERA DNAPrep, San Diego, CA, USA), which we have sequenced in the Illumina NextSeq 1000 equipment (Illumina, San Diego, CA, USA), using FlowCell P1 for obtaining paired-end reads. After running the sequencing, we have processed the obtained raw reads using the Kraken 2 software; human reads were disregarded, and the remaining reads were mapped as reference ON585037 of the monkeypox virus, using the Geneious software (Wellington, New Zealand).<sup>8,9</sup>

**Figura 1.** Number of confirmed cases per epidemiological week (EW), by date of first symptoms, until EW25.



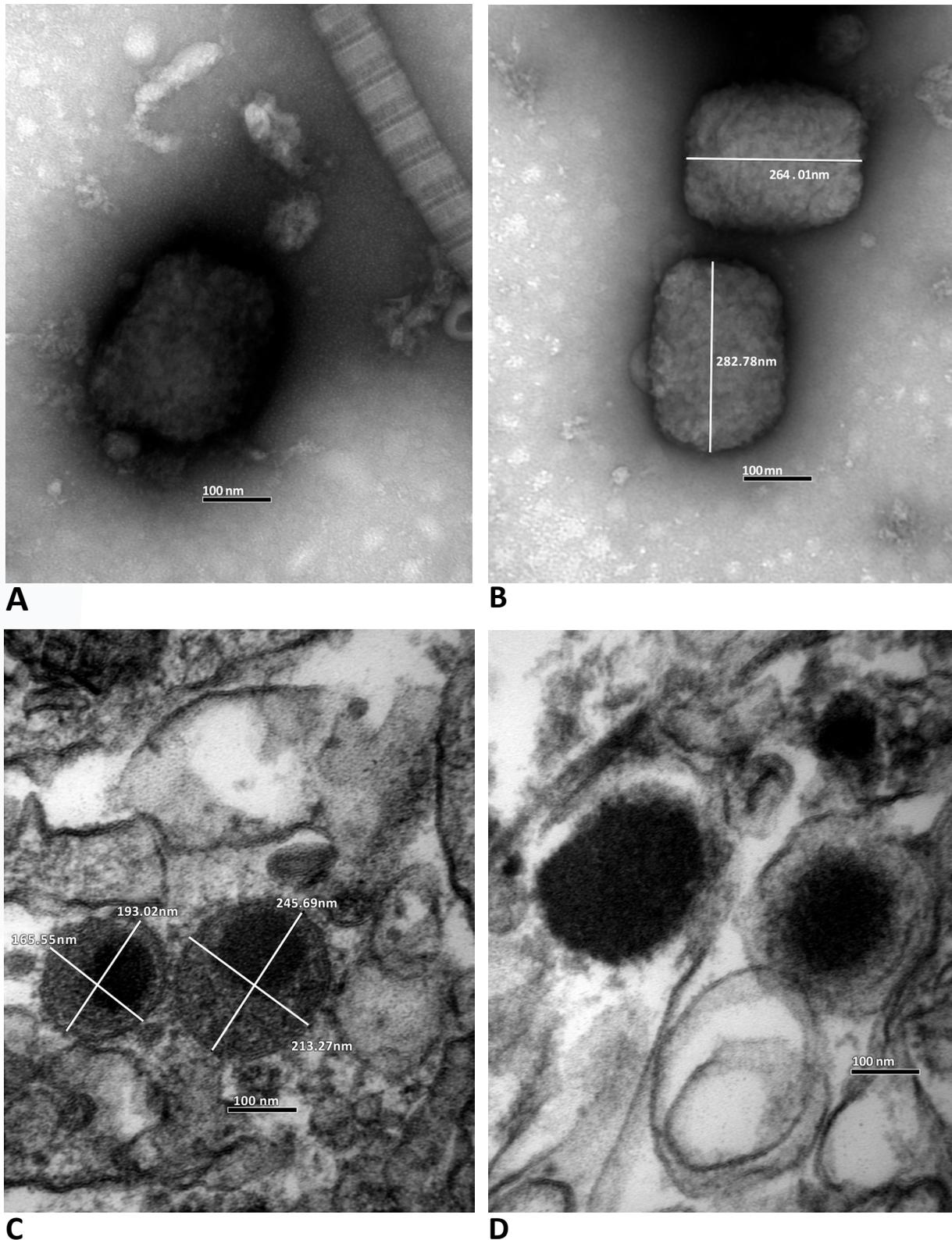
Source: CeVeSP

## RESULTS

São Paulo State confirmed the first case of MPX in June 9th, 2022 (EW22)<sup>6,10</sup>; the EW24 concentrated the highest number of confirmed cases (Figure 1).

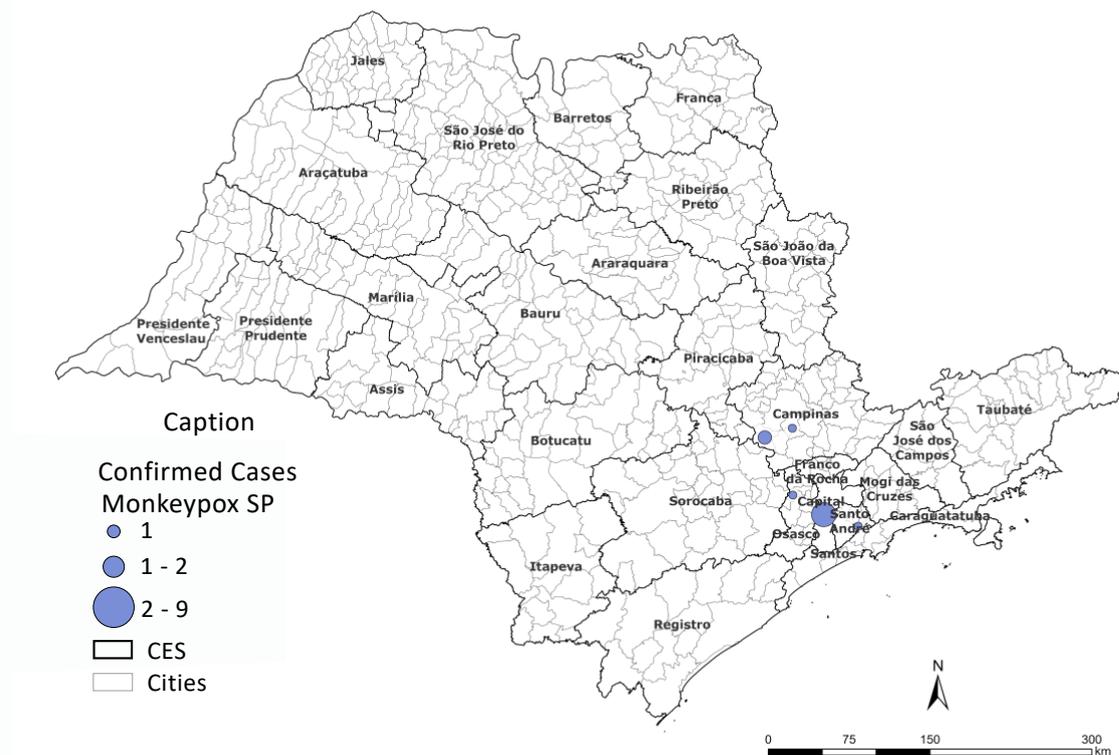
For the first confirmed case in the state<sup>10</sup>, the Electronic Microscopy Center at the Adolfo Lutz Institute received the patient’s lesion biopsy for assessment by electronic microscopy, in order to capture images of the monkeypox virus. Two fragments were sent, one of which was macerated; and, using the liquid from macerating, negative staining was performed. The other fragment was processed for ultra-thin sectioning ([Figure 2](#)).

**Figure 2.** Images captured by electronic microscopy using negative staining (a) and (b); and from ultra-thin sections (c) and (d).



The analysis of data related to the 14 confirmed cases up to June 29th, 2022 showed they were distributed throughout five cities in São Paulo – 64% in the state capital, two cases in Indaiabuba, and one case in Santo André, Itapevi, and Vinhedo (Figure 3).

**Figure 3.** Distribution of confirmed cases of monkeypox in the State of São Paulo up to June 24th, 2022.



Source: CeVeSP

All confirmed cases were male, predominantly referred to as white (78.6%); black and indigenous had one case each. The most affected age group was 25-29 years old (40), and 35-39 years old (4).

As for sexual behavior, 10 cases (71%) reported having multiple partners. They were all men, and reported having sexual relations with men, of which 7 were MSM, and 1 MSMW.

Regarding traveling, 10 cases (71%) reported previous trips abroad—7 to Europe, 1 to the USA, and 2 not informed.

Considering the Adolfo Lutz Institute is the reference laboratory for São Paulo State and all the South Region in Brazil, we have analyzed the complete genomes obtained from the first 13 cases from São Paulo and the 2 confirmed cases from Rio Grande do Sul, comprising around 196,600 base pairs. We have assessed the obtained sequences



## CONCLUSION

Facing the increased number of MPX cases worldwide, the state of São Paulo has managed to structure, in a short period of time, the surveillance over infected people, by implementing notification, and both clinical and laboratorial investigation.

So far, the confirmed cases in the state have the same profile of the current monkeypox outbreak, involving MSM who have a behavior of casual dating multiple partners. It's worth mentioning that any person may be infected, as long as there is contact with the skin of an individual showing lesions.

Nowadays, we acknowledge to be living an outbreak covering extra-endemic countries, where monkeypox has challenged surveillance when it comes to knowledge of clinical, laboratory, and prevention aspects.

We understand that the great challenge to surveillance is to work on risk communication in order to stop the quick spread of the disease, especially in risk groups.

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