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Undetected circulation of monkeypox virus in Portugal: Evidence for a 50-day gap before first detection

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ABSTRACT

As mpox continues to spread globally, proactive monitoring and preparedness are crucial to minimize impact and enhance response strategies. Using a mathematical model combining a negative binomial distribution with Richards' logistic curve, we reconstructed the hidden phase of mpox transmission in Portugal, offering insights into the timing and dynamics of the initial outbreak. The analysis of 950 PCR-positive and 986 negative cases suggested that symptom onset occurred between March 24 and April 2, 2022, with March 27 identified as the most probable date. This study delineates the likely period of silent circulation of MPXV in Portugal, providing a clearer understanding of early outbreak dynamics and surveillance performance. Possible imperfections in early diagnostic testing and limited awareness of mpox may have contributed to delayed recognition of the outbreak. By demonstrating how retrospective mathematical modelling can estimate undetected transmission periods, our findings highlight the value of such approaches in epidemic reconstruction and underscore the importance of strengthening early surveillance systems to detect undiagnosed transmission of mpox in non-endemic countries.

Introduction

Monkeypox virus (MPXV), etiologic agent of mpox, is a member of the *Poxviridae* family, genus *Orthopoxvirus*, which also includes vaccinia, variola (smallpox), and cowpox viruses. These viruses share genetic and antigenic characteristics that contribute to cross-immunity, with historic smallpox vaccination providing partial protection against mpox [1]. MPXV has been classified into two primary clades: clade I (formerly Congo Basin clade), comprising subclades Ia and Ib, and clade II (formerly West African clade), comprising subclades IIa and IIb. Clade I is associated with higher virulence and mortality compared to clade II [1,2].

In May 2022, an unprecedented multi-country outbreak caused by clade IIb strains emerged, prompting the World Health Organization (WHO) to declare a Public Health Emergency of International Concern (PHEIC) in July 2022 [2,3]. A second PHEIC was declared in August 2024 following a rise in clade Ib infections in the Democratic Republic of the Congo and neighbouring countries, with exported cases reported in

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several non-endemic regions [4–6].

By January 2025, over 130,000 confirmed cases of mpox had been reported across more than 130 countries [3]. The scale of this outbreak highlights the need for rapid detection strategies and robust surveillance systems, especially in regions without prior exposure to MPXV [7].

Mathematical modelling has proven valuable in reconstructing epidemic trajectories and in estimating unseen transmission events, such as during early COVID-19 waves [8–12]. For mpox, understanding the timeline of virus introduction and symptom onset is crucial to assess the effectiveness of public health responses.

Previous studies have applied mathematical and statistical modelling to describe the transmission dynamics and detection delays of mpox and other emerging infections. For instance, modelling frameworks have been used to evaluate underdetection and transmission patterns during the 2022–2023 mpox outbreaks [13–15], while other analyses demonstrated how biases in diagnostic accuracy and reporting can distort epidemic curves [16–18]. Also in a recent study, the authors have used SEIR-based model and calculated markedly different model parameters across countries [19]. These findings highlight that early epidemic data are frequently influenced by imperfect diagnostic performance, which may negatively impact on surveillance sensitivity. Building on this evidence, the present study aims to reconstruct the probable onset window of the first mpox infections in Portugal using a hybrid modelling approach. By combining statistical and logistic growth models, we sought not only to estimate the undetected circulation period but also to demonstrate how retrospective modelling can support preparedness and early detection strategies for future outbreaks.

Although Portugal was the second country to confirm mpox cases on May 17, 2022, retrospective analysis suggests that the virus was circulating prior to official detection [20,21]. This study aims to estimate the most probable date of symptom onset for the first mpox case in Portugal using a combined modelling approach. These findings will inform the understanding of the initial transmission window and improve future epidemic preparedness.

Methods

Study population and data processing

This retrospective study analysed laboratory-confirmed mpox cases identified by the Portuguese National Institute of Health (INSA) between April 26 and December 29, 2022. Clinical specimens were collected from suspected mpox patients across all regions of Portugal.

During this period, INSA, as the national reference laboratory, was the only laboratory in the country performing mpox diagnosis, ensuring complete national coverage and methodological consistency. Laboratory confirmation was based on at least one real-time PCR-positive sample per patient, following national guidelines issued by the Directorate-General of Health [21,27]. Suspected cases were reported by healthcare providers through the national surveillance platform (SINAVE), after which clinical and epidemiological data were reviewed by regional public health authorities before official notification. This standardized workflow ensured uniform criteria for laboratory confirmation and case reporting across all regions of Portugal.

Among all suspected cases, 950 were confirmed positive and 986 negative for MPXV. Indeterminate cases ($n = 62$) were excluded. For confirmed cases, both of the sample collection dates and the reported symptom onset dates were reviewed. When discrepancies greater than 20 days were observed between these two dates, the onset date was re-estimated using a probabilistic correction approach to minimize reporting bias.

Mathematical models framework

i) **Negative binomial distribution**

To estimate the distribution of delays between symptom onset and sample collection, a discrete negative binomial distribution was fitted using least squares regression approach. The parameters (n and p) were derived from 626 cases with known onset dates [22]. The probability mass function of the negative binomial distribution is defined as:

$$f(k) = \binom{k+n-1}{n-1} p^n (1-p)^k \quad (1)$$

where k represents the number of days of delay between symptom onset and sample collection, n is the shape parameter describing the number of successful events (or dispersion), and p denotes the probability of observing an additional day of delay.

The negative binomial distribution was chosen due to its suitability for modelling over-dispersed count data, which is common in epidemiological settings where the variance exceeds the mean. In this study, the distribution (eq. 1) was used to estimate the delay between symptom onset and sample collection, a critical step for reconstructing the epidemic curve when direct information is incomplete or biased. Unlike the Poisson distribution, the negative binomial distribution accounts for heterogeneity in individual behaviour, testing practices, and reporting delays, factors that are particularly relevant during emerging outbreaks like mpox. Its flexibility enables a more accurate representation of the empirical delay distribution and supports robust imputation in simulation-based models of transmission dynamics.

ii) **Richards' Growth Model**

A Richards' logistic model, widely used in epidemic forecasting, was employed to generate an epidemic curve based on the moving average (MA) of symptom onset dates and sample dates. The MA was calculated using a 7-day centered window to smooth daily fluctuations and highlight underlying trends. The derivative of the growth function provided the daily case estimates [23–25]. The epidemic curve $I(t)$ (eq. 2) was obtained by the derivative of the accumulated growth curve.

$$I(t) = \frac{rKe^{-r(t-t_m)}}{\left(1+e^{-r(t-t_m)}\right)^2} \quad (2)$$

where, r represents the intrinsic growth rate of the infection, t_m corresponds to the inflection point (the time at which the growth rate changes from accelerating to decelerating), and K denotes the final epidemic size, i.e., the total number of cases expected by the end of the outbreak [10–12]. These parameters were estimated by a least squares regression process using the MA of dates.

The Richards' cumulative growth model was selected due to its superior flexibility in fitting epidemic curves that deviate from the ideal logistic shape. These features are particularly relevant in real-world outbreaks, where underreporting, changes in testing capacity, and behavioural interventions can distort the natural progression of case counts. In the context of the mpox epidemic in Portugal, the Richards' model enabled a more accurate estimation of the inflection point and overall growth trajectory. This provided a robust framework for reconstructing the timeline of early, undetected transmission.

iii) **Simulation approach**

Using the negative binomial distribution, 20,000 simulations were performed to impute symptom onset dates for the 324 cases lacking reliable data. For each simulation, a MA was computed. These MA series were used to fit epidemic curves via least squares, from which prediction confidence intervals were extracted [10–12,26].

iv) **Model calibration and outcome estimation**

The key outcome was the earliest symptom onset date of the putative index case, defined as the lower bound of the 95 % prediction interval

across simulations [26]. The mode of the distribution of all earliest onset estimates was also reported as the most probable date.

Ethics considerations

This study was conducted in accordance with ethical standards and approved by the Ethical Committee for Health of INSA (Approval N° 80/2022). All samples were anonymized, and no personally identifiable data were accessed. MPXV diagnostics were performed under official public health mandates outlined in Technical Orientation N° 004/2022 by the Portuguese Directorate-General of Health [27].

Results

Estimated timeline of first positive cases

The first mpox case in Portugal was officially diagnosed on May 17, 2022 [20]. However, retrospective analysis confirmed that MPXV was already circulating prior to that date [21]. A MA was applied to the daily positive sample collection data to visualize trends. The growth of the MA during this early period indicated a rapid acceleration of infections (Fig. 1A). The first case with a recorded symptom onset reported April 22, 2022 [21].

Determination of stationary MA of positives cases

To identify the most accurate baseline for epidemic curve of positive cases modelling, the ratio between positive and negative tests was analysed over time. This ratio transitioned on May 26, 2022, signalling the point when testing likely began capturing a representative portion of infections in the population. Hence, MA of positives cases prior to this date were not considered stationary for modelling purposes (Fig. 1B).

Delay between symptom onset and sample collection dates

Among the 950 confirmed cases, 626 included symptom onset dates.

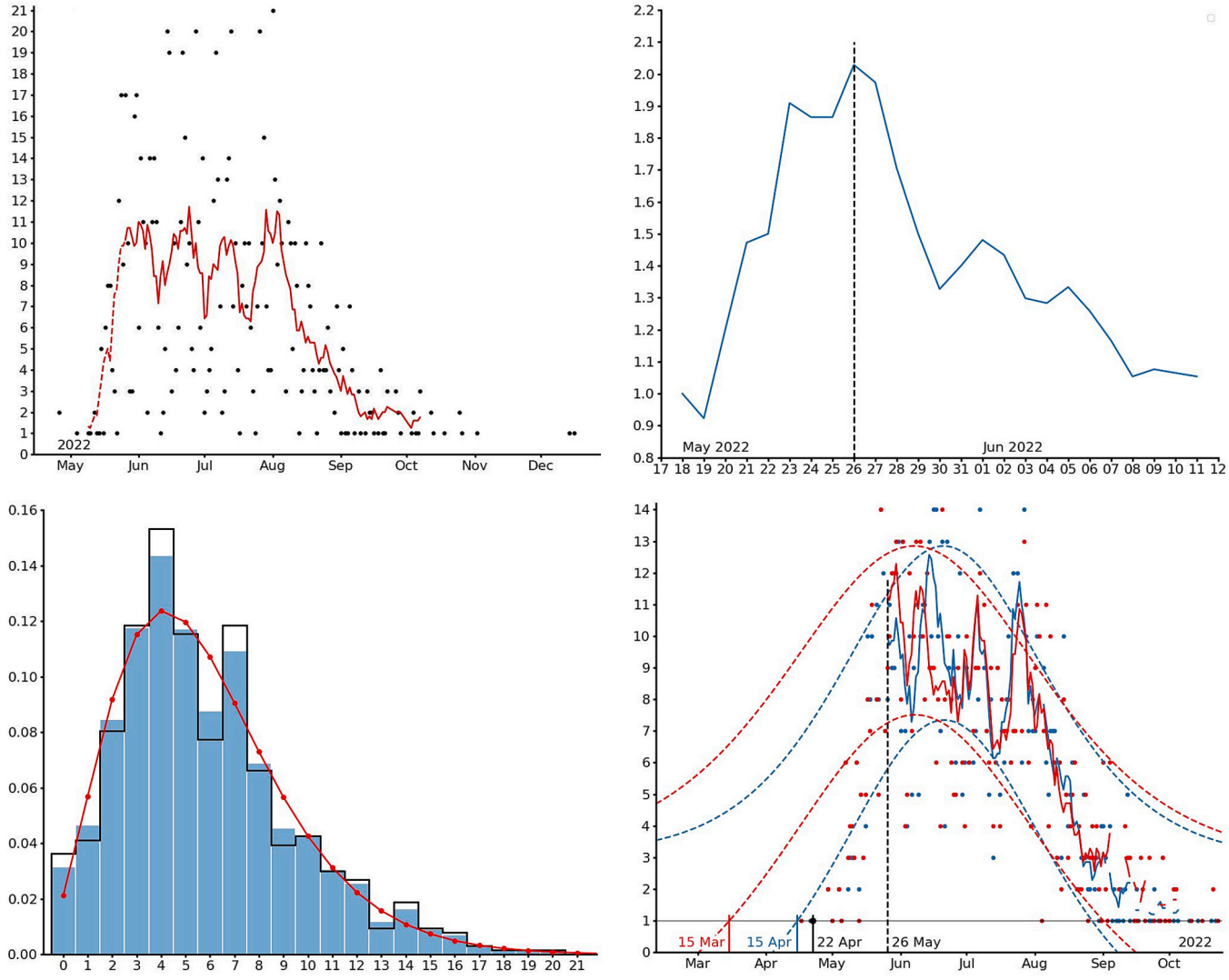

Fig. 1. Evolution of sample collection dates, symptom onset estimates, and modelled epidemic curves. (A) Distribution of positive cases collection dates with MA in 2022 (red line). (B) MA ratio of positive and negative cases, highlighting shift on May 26, 2022, indicating representative population testing. (C) Distribution of delays between sample collection and known symptom onset dates (black line), with fitted negative binomial regression (red line) and simulated distributions from 20,000 imputations (blue bars). (D) Estimated symptom onset dates combining known data and simulated values, with MA starting on May 26 (solid lines); dashed lines indicate the 95 % prediction confidence interval, marking earliest (Mar 15) and latest (Apr 15) onset estimates. (E) Distribution of first symptom onset dates across stationary MA estimates (May 20–26, 2022). (F) Epidemic curve symptom onset dates based on MA starting May 22, showing the most probable onset date (March 27, 2022). Blue line: MA; dashed lines: prediction interval limits; red lines: average limits between simulations with the lower limit on March 27. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

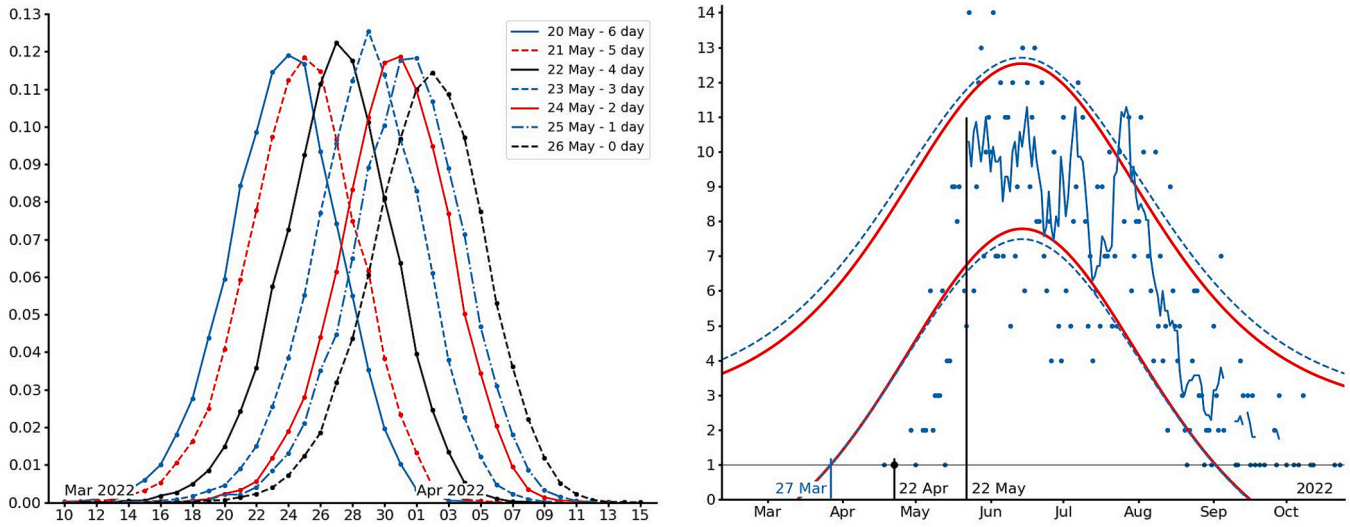

Fig. 1. (*continued*).

The difference between symptom onset and sample collection dates was modelled using a negative binomial distribution, which showed a peak at a 4-day interval (Fig. 1C). The best-fit distribution parameters were $n = 5$ and $p = 0.4627$.

Simulation of unavailable symptoms onset dates

Using the negative binomial distribution, 20,000 simulations were performed to impute symptom onset dates for the 324 positive cases without this information and the respective MA were calculated. Using seven different assumed delays, each MA series was truncated accordingly to create distinct starting points (Table 1). For each MA, an epidemic curve with the respective prediction limits of 95 % was found by least squares regression. Each lower limit of the prediction interval was used to estimate the earliest likely start date.

Using a delay of zero days, we have in Fig. 1D the MA that produces the lower limit of prediction later and the MA that produces the lower limit of prediction earlier, among these we have all the others. All these dates produced a distribution curve with a normal pattern.

All delays have then produced a date distribution curve and the peak of each distribution gave the most likely one. All delays have then produced a distribution curve of most likely dates (Table 1 and Fig. 1E).

Estimated onset window for the first putative case

The most likely dates of symptom onset ranged from March 24 to April 2, 2022 (Table 1). As the most likely delay is four days (Fig. 1C) The most likely onset date was March 27, 2022 (Fig. 1F), implying approximately 50 days of silent viral transmission before the first laboratory-confirmed case. Table 1 summarizes peak onset date estimates for different presumed delay intervals between symptom onset and sample collection dates.

Table 1
Estimated symptom onset dates by assumed delay between symptom onset and sample collection dates.

Delay (days)	MA Start	Onset Range (Start-End)	Peak Onset Date
6	May 20	Mar 10 – Apr 06	Mar 24
5	May 21	Mar 10 – Apr 07	Mar 25
4	May 22	Mar 11 – Apr 09	Mar 27
3	May 23	Mar 13 – Apr 10	Mar 29
2	May 24	Mar 15 – Apr 12	Mar 31
1	May 25	Mar 14 – Apr 14	Apr 01
0	May 26	Mar 15 – Apr 15	Apr 02

Discussion

Portugal was one of the first countries to detect mpox cases on behalf of the 2022 multi-country outbreak and is believed to have played a pivotal role in the initial international spread of MPXV [13]. This study provided strong evidence that the MPXV was circulating in Portugal well before the first laboratory-confirmed case on May 17, 2022 [20,21]. By applying a combined mathematical modelling approach using a negative binomial distribution and Richards' logistic curve, we reconstructed the likely timeline of symptom onset and identified a probable 50-day gap between initial viral transmission and formal detection [22,23].

It is important to recognize that diagnostic accuracy may influence the apparent shape of early epidemic curves. During the initial phase of any emerging outbreak, limited assay validation and variability in testing sensitivity can lead to outcome misclassification, potentially biasing reconstructed trajectories. Similar challenges were documented during the early stages of COVID-19 and influenza pandemics [16–18]. Although our analysis assumes optimal diagnostic performance, undetected false negatives could have slightly extended the estimated period of hidden transmission. Future modelling should incorporate explicit parameters for test sensitivity and specificity to reduce potential bias in the inferred timelines.

The observed 4-day delay between symptom onset and sample collection matches findings from previous viral outbreaks [25]. However, in the early phase of mpox emergence, this gap may have been longer, contributing to delayed recognition. Temporal variation in this interval is expected as public awareness, testing access, and diagnostic capacity evolve throughout the outbreak. Consequently, the delay distribution may have narrowed over time, potentially biasing early epidemic reconstructions. Future models should explicitly incorporate time-dependent delay distributions to account for such dynamics. This temporal heterogeneity may influence the estimated shape of the epidemic curve, potentially leading to underestimation of early transmission intensity if unaccounted for.

The estimation of March 27, 2022, as the most likely date of symptom onset aligns with genomic and epidemiological analyses from other countries affected early in the outbreak [29]. Similar retrospective assessments in the United Kingdom and Spain also suggested silent transmission several weeks prior to case notification, supporting the hypothesis of undetected international spread [7].

While our modelling framework does not assume multiple introductions of MPXV in Portugal in initial stages, our previous study relying in epidemiological and genomic data suggests that multiple importations cannot be entirely excluded [13]. Nevertheless, the limited

phylogenetic diversity in beginning of the Portuguese epidemics, reflecting the dominance of a single lineage, makes it difficult to assess this issue in a robust manner.

Our findings underscore the critical importance of integrating mathematical modelling with epidemiological surveillance [10–12]. Models capable of estimating backdated infection curves allow public health systems to refine their understanding of outbreak origins, improve early response strategies, and calibrate risk communication [10–12]. In Portugal, the combined use of a negative binomial model, to estimate delays between symptom onset and testing, and Richards' growth curve, to reconstruct the epidemic trajectory enabled retrospective estimation of when community transmission likely began. These modelling tools, which have previously proven effective in identifying early, undetected spread during the initial stages of the COVID-19 and SARS outbreaks [10–12,23], were particularly valuable in a context where public health surveillance was not yet attuned to detect a non-endemic pathogen like MPXV [20].

The high proportion of missing symptom onset data (34 % of confirmed cases) is a known limitation in outbreak settings, often due to reporting inconsistencies or asymptomatic presentation. By imputing missing values with probabilistic simulation, we accounted for uncertainty while preserving the integrity of the epidemic curve. The estimates of the present study should be interpreted considering potential uncertainties in retrospective symptom recall and diagnostic delays.

In addition to the combined approach used in this study, other modelling strategies could have been considered. Compartmental models such as SEIR (Susceptible-Exposed-Infectious-Recovered) offer greater granularity by simulating latent periods and the effects of interventions [28]. However, they require detailed epidemiological parameters, which are often unavailable during early outbreak stages or retrospective analyses with incomplete data, as was the case here. Time-series models like ARIMA (Autoregressive Integrated Moving Average) are useful for short-term trend forecasting but lack the epidemiological foundations needed for robust back-estimation of undetected cases [29]. Hierarchical Bayesian models offer strong flexibility by incorporating uncertainty and prior knowledge, yet they demand significant computational resources and calibration effort [30]. The hybrid approach adopted here, combining a negative binomial distribution with Richards' growth model, proved particularly well-suited to the study objectives. This methodology has previously demonstrated its reliability in reconstructing hidden transmission phases during the early waves of COVID-19 and SARS, especially in settings where detection was delayed. Its successful application across diverse outbreaks reinforces its value in retrospective modelling, particularly when dealing with delayed detection and missing symptom data [10–12].

Our analysis is subject to limitations. First, the negative binomial model assumes a stable distribution of delays, which may not reflect dynamic testing access or behaviour changes during the outbreak described by others [22,26]. Second, symptom onset data are subject to recall bias, especially in retrospective collection. Third, the model does not account for asymptomatic transmission or underreporting in subclinical cases. Regarding the latter, a previous study estimated a 62 % infection-reporting rate in Portugal [14]. Despite these constraints, the overall trajectory observed, progressing from undetected introduction to confirmed community spread, matches the profile of other imported epidemics [11,15]. Moreover, the persistence of clade IIb in Portugal, in the absence of clade I introductions, may reflect specific transmission dynamics and travel patterns [29,31].

Beyond reconstructing past transmission dynamics, the modelling approach presented here provides actionable insights for strengthening public health surveillance systems. By identifying the likely window of undetected transmission, such retrospective analyses highlight critical periods when enhanced diagnostic capacity, public awareness, or targeted testing could have accelerated case recognition. Integrating real-time modelling tools that account for diagnostic accuracy and reporting delays into national surveillance platforms, such as SINAVE, could enable earlier detection of anomalous transmission patterns and improve situational awareness during emerging outbreaks. These insights also support preparedness planning for other re-emerging or novel pathogens, particularly in non-endemic regions where early warning signals are easily overlooked. Such retrospective analyses can inform prospective surveillance by identifying diagnostic and reporting delays that affected early detection during the mpox outbreak. Recognizing these gaps allows public health systems to adjust alert thresholds, refine case definitions, and expand testing capacity more rapidly in future events, thereby reducing the time between initial transmission and detection.

In conclusion, this study demonstrates the silent circulation of mpox in Portugal for nearly 50 days before official detection, emphasizing the need for early and adaptive surveillance in non-endemic regions. Mathematical modelling proved essential for reconstructing hidden transmission phases. Strengthening laboratory-based detection, timely data reporting, and integrated public health responses will be critical for preventing future outbreaks and improving global preparedness against emerging infectious diseases.

CRediT authorship contribution statement

Rita Cordeiro: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Fernando da Conceição Batista:** Writing – original draft, Validation, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Ana Pelerito:** Writing – review & editing, Investigation. **Isabel Lopes de Carvalho:** Writing – review & editing, Investigation. **Sílvia Lopo:** Writing – review & editing, Investigation. **Raquel Neves:** Writing – review & editing, Investigation. **Raquel Rocha:** Writing – review & editing, Investigation. **Paula Palminha:** Writing – review & editing, Investigation. **Maria José Borrego:** Writing – review & editing, Investigation. **Maria Sofia Núncio:** Writing – review & editing, Investigation. **João Paulo Gomes:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT (GPT-5 model, OpenAI, accessed via https://chat.openai.com) in order to assist in language editing, paragraph restructuring, and summarization. After using this tool/service, the author(s) reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of competing interest

None.

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