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RESEARCH LETTER

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Isolation and genomic characterization of Chikungunya virus during the 2025 outbreak in Foshan, China

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ABSTRACT

Chikungunya virus (CHIKV) caused more than 10,000 infections in Foshan, Guangdong, in 2025. Using plasma from acute-phase patients, we successfully isolated infectious CHIKV, confirmed by focus-forming assay and transmission electron microscopy. Whole-genome sequencing revealed that all isolates belonged to the ECSA-2 sub-lineage and were closely related to strains circulating on Réunion Island in 2024–2025, carrying adaptive mutations including C:T143A, E1:A226 V, and E2:I211 T/L210Q. Based on Foshan's 2025 population (~9.6 million), the outbreak corresponded to a cumulative incidence of ~104 cases per 100,000 inhabitants, highlighting intense transmission. Integrated virological, genomic, and epidemiological analyses characterize the rapid expansion of a single ECSA-2 genotype and underscore the need for strengthened arbovirus and genomic surveillance in southern China.

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Dear editor,

Chikungunya virus (CHIKV), an alphavirus of the family *Togaviridae*, is an emerging arbovirus transmitted mainly by *Aedes aegypti* and *Aedes albopictus* [1]. CHIKV infection causes acute febrile illness with debilitating polyarthralgia and has triggered explosive outbreaks across multiple continents, including Africa, Asia, and the Americas [2]. Three major genotypes are recognized: the West African, the Asian, and the East/Central/South African (ECSA) lineages, each associated with distinct geographic distributions and epidemic histories [3]. Since the mid-2000s, the ECSA lineages have expanded from Africa into the Indian Ocean region, Asia, and the Americas, often accompanied by adaptive mutations such as E1:A226 V that enhance transmission by *Aedes albopictus* [4, 5]. The ECSA lineage has been responsible for several recent epidemics, highlighting its global epidemic potential [6, 7]. Although molecular diagnostics are widely adopted, isolation and characterization of infectious virus remains essential to public health surveillance [8, 9].

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Prior to 2025, Guangdong Province reported only sporadic CHIKV cases, with no confirmed local transmission in Foshan. In 2025, Foshan recorded more than 10,000 cases during July–August [10]. The outbreak, initially emerging in Shunde District, rapidly expanded across Foshan, with a high basic reproduction number ($R_0 = 16.3$) [11]. With an estimated population of ~9.6 million inhabitants, this corresponds to a cumulative incidence of ~104 cases per 100,000, signalling unusually intense and rapid viral transmission. These epidemiological indicators provide essential context for interpreting the laboratory and genomic findings described below.

Serum exhibited the highest CHIKV detection rate (90%) and viral load, defining it as the optimal specimen type; viral RNA peaked during days 0–7 post-onset, marking the ideal diagnostic window [12]. Most patients presented with fever, rash, and arthritis, consistent with typical chikungunya manifestations. To investigate the circulating strains, we attempted virus isolation from serum samples collected at two local hospitals, including Foshan Shunde Third People's Hospital (Beijiao Hospital) and the Second People's Hospital of Foshan. Four RT-qPCR–positive patients with high viral loads (Ct: 16–20) were selected. Plasma samples were inoculated onto *Aedes albopictus* C6/36 cell monolayers in a BSL-3 facility. Cytopathic effects, including cell rounding and detachment, appeared on day 4–5 post-inoculation, and CHIKV RNA in culture supernatants was confirmed by RT-qPCR. All four samples yielded infectious virus, with cytopathic effects observed by days 4–5 (Figure 1A), reflecting the high viremia characteristic of acute chikungunya and demonstrating that serum-based virological surveillance is feasible during early outbreak phases.

To verify that the isolates were infectious in susceptible cells, fresh Vero E6 cells were inoculated and analyzed by focus-forming assay (FFA) with a CHIKV E2-specific rabbit polyclonal antibody. Clear immunostaining was detected in infected cells but absent in mock controls (Figure 1B). To further validate successful viral isolation, CHIKV particles were inactivated with paraformaldehyde and examined by transmission electron microscopy (TEM). TEM revealed typical spherical, enveloped CHIKV particles with surface projections (~60 nm in diameter), consistent with alphavirus morphology (Figure 1C). In addition, some particles lacking envelopes were observed, which may result from membrane loss during paraformaldehyde inactivation and sample preparation. These results provide virological confirmation of circulating strains and directly inform laboratory preparedness during emerging outbreaks.

To further determine the genotype and mutational features of the circulating strains, we performed whole-genome phylogenetic and single amino acid polymorphism analyses. Full-length genomes (~11.7 kb) were obtained from all four isolates using targeted next-generation sequencing (tNGS) with the CHIKV Genome Sequencing Kit. Sequence comparison showed >99% nucleotide identity, and phylogenetic analysis clustered all four strains within the ECSA-2 sub-lineage (Figure 1D), closely related to strains recently detected on Réunion island during 2024-2025. Notably, these viruses carried signature mutations including C:T143A, E1:A226 V, and E2: I211 T/L210Q, which have been linked to enhanced transmission by *Aedes albopictus* (Figure 1D). These genomic traits help explain the rapid expansion of the outbreak and illustrate how early identification of vector-adapted lineages can strengthen public health surveillance.

To further assess the genetic consistency of the outbreak strain, we sequenced additional clinical samples and obtained 34 partial or near-complete genomes. Phylogenetic and SNP analyses showed high concordance with the four cultured isolates (Figure S1), indicating that the CHIKV strains associated with this outbreak were highly homogeneous and belonged to a single ECSA-2 lineage. This pattern indicates a single introduction followed by rapid local amplification rather than long-term undetected circulation, and demonstrates the value of genomic surveillance in differentiating introduction events from persistent endemic transmission.

In conclusion, the chikungunya outbreak in Foshan, Guangdong, in 2025 was driven by a single circulating ECSA-2 genotype that expanded rapidly within the population. Although the precise determinants underlying its transmissibility and pathogenicity remain to be fully elucidated, the pathways of introduction and subsequent transmission dynamics warrant further investigation. Through successful viral isolation, ultrastructural confirmation, and whole-genome characterization, our study provides essential baseline data for understanding this outbreak. These findings offer molecular and morphological evidence of ECSA-2 circulation and emphasize the importance of arbovirus isolation capacity and genomic surveillance for enhancing outbreak preparedness and response.

This study has several limitations. The number of viral isolates and sequenced samples was modest compared with the >10,000 reported cases during the outbreak, and therefore may not capture the full extent of viral diversity or minor variants. Moreover, the absence of entomological surveillance data limits our ability to assess vector infection dynamics or environmental drivers. Nonetheless, the consistent genomic clustering observed across all available samples supports the robustness of our inference that the outbreak was driven by a single, rapidly spreading ECSA-2 lineage.

A

CHIKV patients	Collection time	Sample type	Ct value (target: E1)	NCBI accession No.
P1	20250724	plasma	17	PX216391
P2	20250724	plasma	20	PX216392
P3	20250727	plasma	18	PX216393
P4	20250731	plasma	17	PX216394

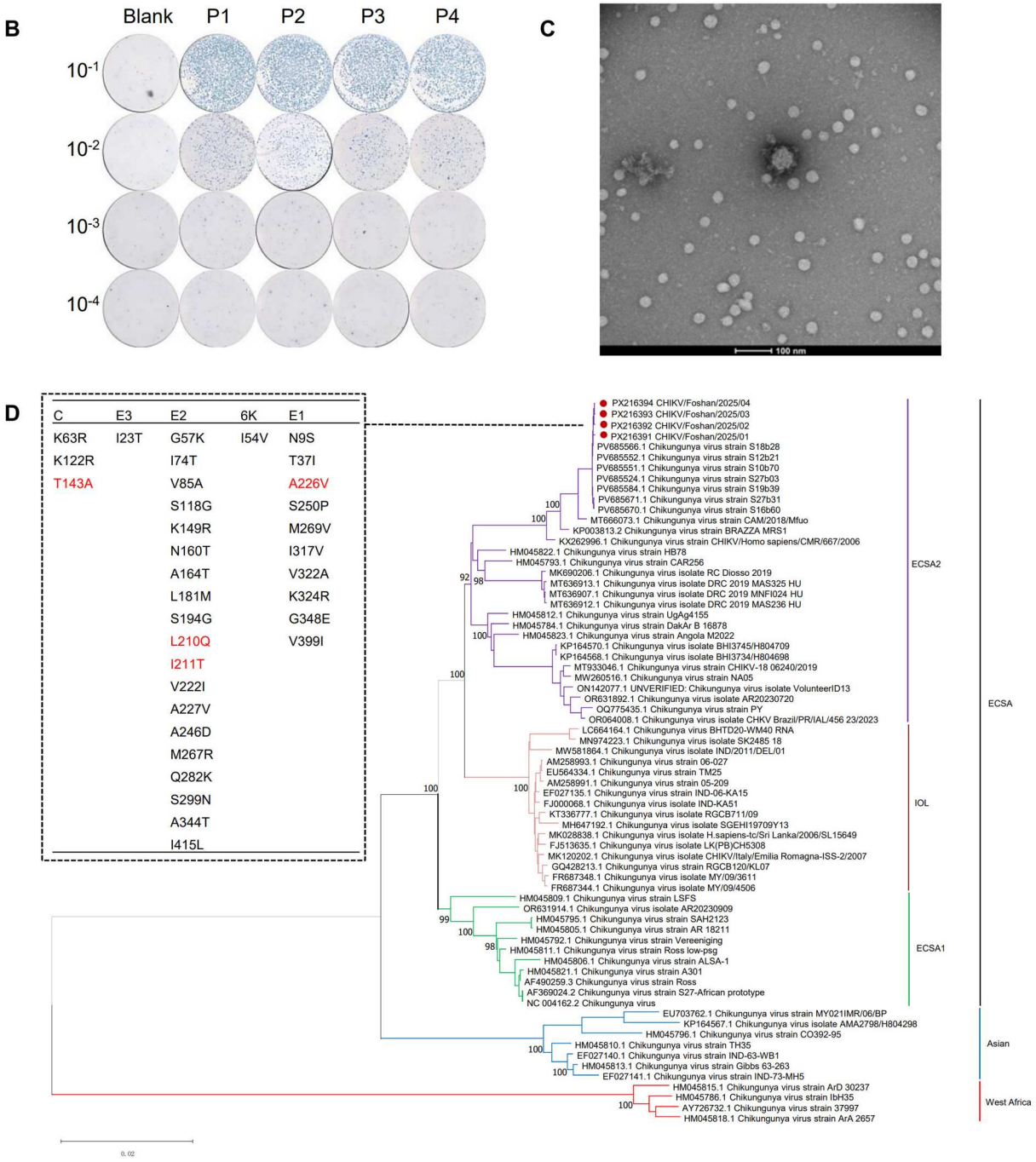

Figure 1. Isolation and genomic phylogeny of imported Chikungunya Viruses in Foshan, China, 2025 (A) Overview of CHIKV cases in Foshan, Guangdong in 2025 and whole-genome sequences obtained in this study. (B) Detection of CHIKV infection in Vero E6 cells by focus-forming assay (FFA). Vero E6 cells were inoculated with virus-containing supernatant for 24 h, and viral proteins were detected with a CHIKV E2-specific rabbit polyclonal antibody. (C) Visualization of viral particles using Transmission Electron Microscopy (TEM). (D) Phylogenetic relationship analysis and single amino acid comparison were performed. The complete genomes of 65 representative strains of CHIKV in GenBank were analyzed using the maximum-likelihood method implemented in MEGA version 11.0. Numbers at the nodes represent bootstrap support. Single amino acid comparison of structural proteins between CHIKV reference strain (NC_004162.2) and Foshan epidemic strain (CHIKV/Foshan/2025/01) obtained in this study was performed.

Contributors

Baisheng Li, Jincun Zhao, Yanqun Wang and Lifen Huang conceived and designed the study. Changyun Sun, Lu Zhang, Haisu Yi, Shuichun Wan, Airu Zhu and Bin Yuan collected clinical specimens and performed the experiments. Qihong Yan,Xiaofang Peng, Chao Yu, Xing Zhang, Kangkang Fu, Jingxiong Zeng and Yongxia Shi analyzed the data. All authors contributed to manuscript writing and approved the final version.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Ethics statement

Approval from the Medical Research Ethics Committee of the Guangdong Provincial Center for Disease Control and Prevention under approval number W96-027E-202516.

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