3. Simón F, Siles-Lucas M, Morchón R, González-Miguel J, Mellado I, Carretón E, et al. Human and animal dirofilariasis: the emergence of a zoonotic mosaic. Clin Microbiol Rev. 2012;25:507–44. https://doi.org/10.1128/CMR.00012-12
4. Colella V, Young ND, Manzanell R, Atapattu U, Sumanam SB, Huggins LG, et al. *Dirofilaria asiatica* sp. nov. (Spirurida: Onchocercidae)—defined using a combined morphological-molecular approach. Int J Parasitol. 2025;55:461–74. https://doi.org/10.1016/j.ijpara.2025.04.006
5. Orihel TC, Ash LR. Parasites in human tissues. Chicago: ASCP Press; 1995.
6. Mulanovich EA, Mulanovich VE, Rolston KVI. A case of *Dirofilaria* pulmonary infection coexisting with lung cancer. J Infect. 2008;56:241–3. https://doi.org/10.1016/j.jinf.2008.01.007
7. Spratt DM. Histological morphology of adult *Dirofilaria roemeri* and anatomy of the microfilaria. Int J Parasitol. 1972;2:193–200. https://doi.org/10.1016/0020-7519(72)90006-9
8. Theodore SG, Sawkins HJ, Mathew M, Yadav S, Norton R. Human pulmonary dirofilariasis: an unexpected differential diagnosis for a solitary lung lesion. Med J Aust. 2023;219:455–6. https://doi.org/10.5694/mja2.52115
9. Constantinoiu C, Croton C, Paterson MBA, Knott L, Henning J, Mallyon J, et al. Prevalence of canine heartworm infection in Queensland, Australia: comparison of diagnostic methods and investigation of factors associated with reduction in antigen detection. Parasit Vectors. 2023;16:63. https://doi.org/10.1186/s13071-022-05633-9

Address for correspondence: Richard Bradbury, PHTM, Building 41, James Cook University, 1 James Cook Dr, Townsville, QLD 4814, Australia; email: richard.bradbury@jcu.edu.au

Detection of and Early Genomic Insights into Chikungunya Virus, Bolivia, 2025

Joel Alejandro Chuquimia Valdez,[1] Natalia R. Guimarães,[1] Vagner Fonseca,[1] Cleidy Orellana Mendoza, Sebastián Sasías Martínez, Sara Cândida F. Santos, Gilson Carlos Soares, Mariela Martínez Gómez, Leticia Franco, Lionel Gresh, Jairo Méndez-Rico, Luiz Carlos J. Alcantara,[2] Marta Giovanetti,[2] Leidy Roxana Loayza Mafayle[2]

Author affiliations: Centro Nacional de Enfermedades Tropicales, Santa Cruz de la Sierra, Bolivia (J.A.C. Valdez, C.O. Mendoza, S.S. Martinez, L.R. Loayza Mafayle); Fundação Ezequiel Dias, Belo Horizonte, Brazil (N.R. Guimarães, S.C.F. Santos); Instituto René Rachou, Belo Horizonte (N.R. Guimarães, S.C.F. Santos, L.C.J. Alcantara); Universidade do Estado da Bahia, Salvador, Brazil (V. Fonseca); Stellenbosch University, Stellenbosch, South Africa (V. Fonseca); Universidade Federal de Minas Gerais, Belo Horizonte, (S.C.F. Santos, G.C. Soares); Pan American Health Organization, Washington, DC, USA (M. Martínez Gómez, L. Franco, L. Gresh, J. Méndez-Rico); Università Campus Bio-Medico di Roma, Rome, Italy (M. Giovanetti); Instituto Oswaldo Cruz, Rio de Janeiro, Brazil (M. Giovanetti).

DOI: https://doi.org/10.3201/eid3207.260540

We report the detection and genomic characterization of chikungunya virus, an arbovirus, during a 2025 outbreak in Bolivia. We identified the circulating chikungunya virus lineage and the transmission dynamics by using genomic surveillance and phylogenetic analyses. Our findings highlight the utility of sustained genomic surveillance for monitoring emerging arboviruses.

Chikungunya virus (CHIKV) is a positive-sense RNA virus belonging to the genus *Alphavirus* (family Togaviridae), primarily transmitted by *Aedes aegypti* and *A. albopictus* mosquitoes. CHIKV is comprised of 3 major lineages: West African, Asian, and East/Central/South African (ECSA). The Asian lineage was introduced into the Americas in 2013, and the ECSA lineage was introduced in 2014. Those introductions gave rise to the Asian-American and ECSA-American sublineages (*1*). Chikungunya infection is typically characterized by acute febrile illness with polyarthralgia, although severe manifestations, including neurologic complications, can occur (*1*). Globally, CHIKV has expanded greatly, with an estimated 16.9 million cases annually and >5.6 billion persons living in at-risk areas (*1*). The Asian-American lineage was first detected in Bolivia in 2015, followed by outbreaks in 2016 and 2017 (*2*). In 2025, a major CHIKV outbreak took place in Bolivia after several years without any reported cases. That outbreak included 4,696 confirmed cases, and most cases (90.8%) were in Santa Cruz (*3*). This resurgence highlights the vulnerability of previously affected regions to new CHIKV outbreaks and underscores the need for sustained surveillance.

This work is part of the routine arbovirus genomic surveillance implemented in Bolivia. Samples used in this study were obtained anonymously from

[1]These first authors contributed equally to this article.
[2]These senior authors contributed equally to this article.

material exceeding routine arbovirus diagnostics within Bolivia's public health laboratory network. To investigate the origin and transmission dynamics of the 2025 outbreak, we implemented genomic surveillance of CHIKV in Bolivia. We selected 78 quantitative reverse transcription PCR–positive samples (cycle threshold [Ct] ≤30), collected from February–June 2025 from 4 departments (Chuquisaca, Cochabamba, Santa Cruz, and Tarija) for our analysis on the basis of Ct value and available metadata (Figure 1).

We used a multiplex PCR approach to amplify CHIKV RNA (*4*), and we sequenced CHIKV by using Illumina (Illumina, https://www.illumina.com) and Oxford Nanopore (Oxford Nanopore, https://nanoporetech.com) platforms. We generated consensus genomes by using combined de novo and reference-based approaches (*5*). We conducted a phylogenetic analysis by using genomes from the 78 selected samples together with the 972 publicly available ECSA sequences from the National Center for Biotechnology Information database, which included complete sequences, sampling date, and geographic origin. We performed multiple sequence alignment by using MAFFT (*6*), and we inferred maximum-likelihood phylogenies by using IQ-TREE (*7*). We assessed temporal signal by using root-to-tip regression, yielding a correlation coefficient of 0.52, consistent with sufficient temporal structure for molecular clock inference (*8*). We performed time-scaled phylogeographic reconstruction by using BEAST (*9*) under a relaxed molecular clock model, with an estimated mean evolutionary rate of 2.18 × 10^{-3} substitutions/site/year.

Samples were collected from patients 0–90 years of age, with the highest proportion of samples from patients 0–9 years of age (19.2%, n = 15), followed by samples from patients 20–29 years of age (16.7%, n = 13), and 30–39 years of age (16.7%, n = 13). Most (52.6%, n = 41) samples were from female patients (Appendix 1 Figure 1, http://wwwnc.cdc.gov/EID/article/32/7/26-0540-App1.pdf; Appendix 2 Table, http://wwwnc.cdc.gov/EID/article/32/7/26-0540-App2.xlsx). Most patients had acute febrile illness (n = 60), and 18 cases were classified as severe, including 1 encephalitis case and 1 fatal outcome (Appendix 1 Figure 2).

The sequenced samples had Ct values ranging from 10 to 28 (mean 18.5) (Appendix 1 Figure 3). Sequencing generated 78 near-complete CHIKV genomes with an average genome coverage of 95.9%. All genomes were classified as the ECSA lineage. All CHIKV genomes from Bolivia formed a well-defined monophyletic clade, with the closest ancestry linked to viruses circulating in Midwest Brazil

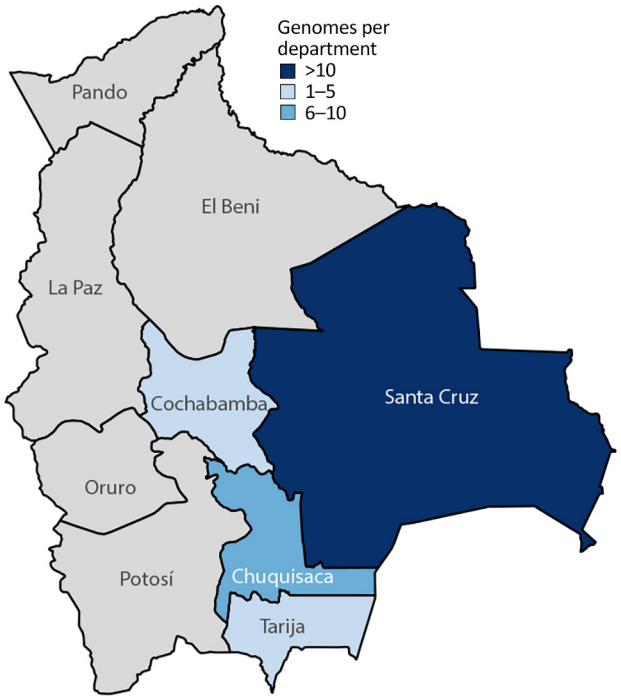

Figure 1. Geographic distribution of sequenced chikungunya virus genomes in a study of chikungunya virus in Bolivia, 2025. Colors indicate the numeric range of genomes per department.

(Figure 2, panel A). This genomic analysis suggests that the 2025 CHIKV outbreak in Bolivia was driven by a single introduction event followed by sustained local transmission. Time-scaled phylogeographic analysis estimated CHIKV introduction around November 2024 (95% CI late October–early November). The earliest transmission was inferred in Chuquisaca, followed by dissemination to Santa Cruz and subsequent spread to Tarija and Cochabamba (Figure 2, panel B). The inferred directional spread toward more densely populated regions further supports the role of human mobility and urban transmission networks in enabling rapid geographic expansion (*10*).

Our results reveal the genetic similarity of CHIKV strains circulating in Bolivia during the 2025 outbreak and provides evidence indicating a single introduction of CHIKV from Midwest Brazil, with subsequent spread across multiple departments. Our findings improve our knowledge of CHIKV transmission dynamics in Bolivia; however, limitations in temporal and geographic sampling coverage might have limited full characterization of viral diversity. Our findings also demonstrate how integrating genomic surveillance into outbreak investigations enables identification of introduction events and reconstruction of transmission pathways, providing critical insights to inform public health interventions. Because of increasing arboviral activity across the Americas,

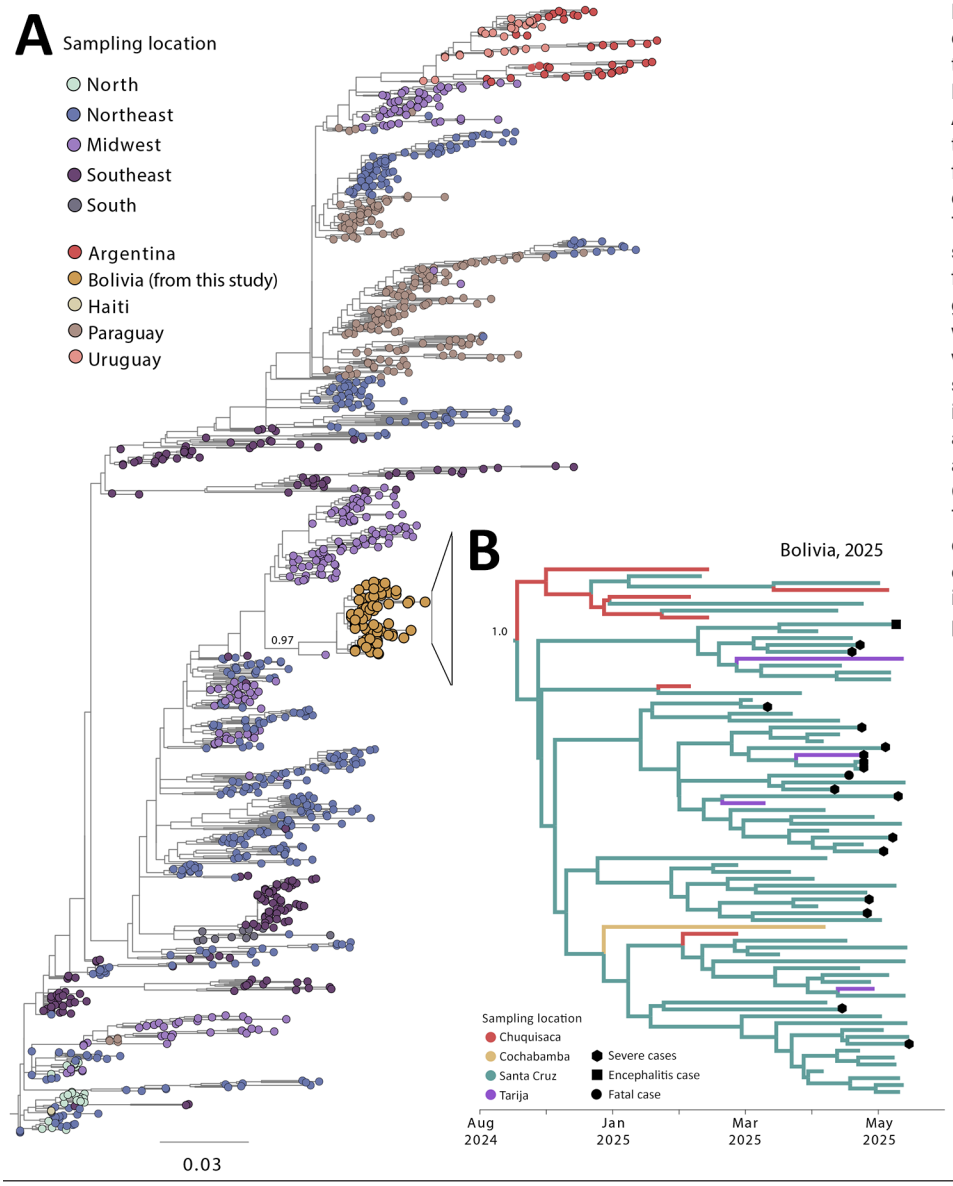

Figure 2. Regional genetic diversity and temporal spread of the chikungunya virus outbreak in Bolivia within the Americas, 2025. A) Phylogenetic tree showing the placement of genomes from Bolivia within the broader diversity across the Americas. Tips are colored according to sampling origin, and sequences from Bolivia are highlighted. The genomes from Bolivia cluster in a well-supported group, consistent with local expansion. B) Time-scaled tree of the Bolivian clade illustrating temporal progression and geographic distribution across departments (Chuquisaca, Cochabamba, Santa Cruz, and Tarija). Symbols indicate severe cases (including 1 encephalitis case and 1 fatal case). Scale bar indicates nucleotide substitutions per site.

those approaches are essential to improve early detection and guide timely response strategies.

Acknowledgments

We thank Dina Condori Choque and Alinda Nilda Espinoza Quevedo for their support in providing epidemiological data at the national level. We thank the departmental reference laboratories of Cochabamba, Tarija, and Chuquisaca for collecting and sending serum samples for molecular diagnosis and genomic surveillance of chikungunya to the National Center for Tropical Diseases.

The XML file (https://github.com/genomicsurveillance/chikv_bolivia) and raw sequencing data and associated metadata (BioProject accession no. PRJNA1460444) are available.

N.R.G. was supported partially by the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (project no. RED-00234-23). V.F. was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil (grant nos. 444903/2024-0 and 314941/2025-8). This work was supported by the Centers for Disease Control and Prevention award (cooperative agreement no. CK000639) to the Pan American Health Organization (PAHO), by the Novo Nordisk Foundation (award no. NNF24OC0094346), and by the Pandemic Response Optimization Through Engaged Communities and Territories project. PROTECT project, led by 7 countries including Bolivia, is supported by a grant from the Pandemic Fund, a multilateral financing mechanism dedicated exclusively to strengthening pandemic prevention, preparedness, and response capacities

in low- and middle-income countries. Project implementation is supported by 2 implementing entities: PAHO and the World Bank.

About the Author

Dr. Valdez is a researcher at the Centro Nacional de Enfermedades Tropicales (CENETROP) in Santa Cruz, Bolivia, where he is responsible for genomic surveillance activities.

Reference

1. de Souza WM, Lecuit M, Weaver SC. Chikungunya virus and other emerging arthritogenic alphaviruses. Nat Rev Microbiol. 2025;23:585–601. https://doi.org/10.1038/s41579-025-01177-8
2. França CMB, Loayza R, Roca Y, Montaño Arias AM, Tinajeros F, Loaiza JR, et al. Genome sequences of chikungunya virus isolates from Bolivia. Microbiol Resour Announc. 2020;9:e00230–20. https://doi.org/10.1128/MRA.00230-20
3. Pan American Health Organization. Epidemiological alert: chikungunya. 2026 [cited 2026 Mar 27]. https://www.paho.org/en/documents/epidemiological-alert-chikungunya-10-february-2026
4. Quick J, Grubaugh ND, Pullan ST, Claro IM, Smith AD, Gangavarapu K, et al. Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. Nat Protoc. 2017;12:1261–76. https://doi.org/10.1038/nprot.2017.066
5. Vilsker M, Moosa Y, Nooij S, Fonseca V, Ghysens Y, Dumon K, et al. Genome Detective: an automated system for virus identification from high-throughput sequencing data. Bioinformatics. 2019;35:871–3. https://doi.org/10.1093/bioinformatics/bty695
6. Katoh K, Standley DM, Yamada KD. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30:772–80. https://doi.org/10.1093/molbev/mst010
7. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 2015;32:268–74. https://doi.org/10.1093/molbev/msu300
8. Rambaut A, Lam TT, Max Carvalho L, Pybus OG. Exploring the temporal structure of heterochronous sequences using TempEst (formerly Path-O-Gen). Virus Evol. 2016;2:vew007. https://doi.org/10.1093/ve/vew007
9. Baele G, Ji X, Hassler GW, McCrone JT, Shao Y, Zhang Z, et al. BEAST X for Bayesian phylogenetic, phylogeographic and phylodynamic inference. Nat Methods. 2025;22:1653–6. https://doi.org/10.1038/s41592-025-02751-x
10. Chadsuthi S, Althouse BM, Iamsirithaworn S, Triampo W, Grantz KH, Cummings DAT. Travel distance and human movement predict paths of emergence and spatial spread of chikungunya in Thailand. Epidemiol Infect. 2018;146:1654–62. https://doi.org/10.1017/S0950268818001917

Address for correspondence: Marta Giovanetti, Fundação Oswaldo Cruz (Fiocruz), Av. Brasil 4365, Manguinhos, Rio de Janeiro, RJ 21040-900, Brazil; email: giovanetti.marta@gmail.com

Ophthalmomyiasis Outbreak Caused by *Oestrus ovis* Infection, Algeria, 2025

Yuyang Zeng,[1] Hongkuan Yang,[1] Xin Li, Hongzheng Yang, Yunyun Zhou

Author affiliations: Spencer Center for Vision Research Department of Ophthalmology, Byers Eye Institute at Stanford University School of Medicine, Palo Alto, California, USA (Y. Zeng); Renmin Hospital of Wuhan University Research Department of Ophthalmology, Wuhan, China (Y. Zeng, Y. Zhou); Tongji Hospital of Huazhong University of Science and Technology Department of Anesthesiology, Wuhan (Hongkuan Yang); Hubei Cancer Center Hospital of Huazhong University of Science and Technology Department of Anesthesiology, Wuhan (X. Li); Renhe Hospital Affiliated with China Three Gorges University Department of Medical Cosmetology, Yichang, China (Hongzheng Yang)

DOI: https://doi.org/10.3201/eid3207.260552

Ophthalmomyiasis is a rare eye infestation caused by fly larvae and more often seen in rural areas. We report an outbreak of 17 patients in Algeria with ophthalmomyiasis after sheep exposure. All patients fully recovered after removal of ocular *Oestrus ovis* larvae and topical therapy, highlighting the effectiveness of early detection and treatment.

Ophthalmomyiasis is a rare ocular infestation in modern clinical settings. *Oestrus ovis*, the sheep nasal bot fly, is the most common cause of human cases (*1*). Because *O. ovis* larvae primarily infect sheep and goats, human infection occurs predominantly in rural settings, although urban cases have been reported (*2*–*4*). Ophthalmomyiasis is classified as external, internal, or orbital, on the basis of infestation site. External ophthalmomyiasis is limited to the ocular surface, involving the conjunctiva and cornea (*5*). Internal ophthalmomyiasis affects intraocular structures including the anterior chamber, choroid, and vitreous (*6*). Last, orbital ophthalmomyiasis involves the orbital cavity and adjacent tissues. Larval migration and intraocular involvement can cause structural damage and vision loss (*7*).

We report a case series of 17 patients with acute external ophthalmomyiasis caused by *O. ovis* infection after sheep exposure during ritual sacrifice for Eid al-Adha in Algeria during June 6–8, 2025. Patients were 26–45 years of age; 10 were men and 7 were women.

[1]These first authors contributed equally to this article.