Mayaro Virus Emergence and Genetic Dynamics, Peru

Lester J. Perez, Austin Hodges, Katherine Torres, Kenn Forberg, Sonja Weiss, Julie Yamaguchi, Maximillian Mata, Alonso Cruz-Echevarría, Diana Cubas, Freddy Alava, Dionicia Gamboa, Joseph M. Vinetz, Michael G. Berg, Gavin A. Cloherty

Mayaro virus (MAYV) is an emerging pathogen in Latin America. Using phylodynamic reconstructions and phylowave analyses, we describe novel lineage diversification and rapid dispersal of MAYV in Peru. A newly developed, dual-target quantitative reverse transcription PCR assay reliably detects MAYV variants, enabling improved surveillance, control, and understanding of this neglected arbovirus.

Mayaro virus (MAYV) is an endemic, neglected, zoonotic alphavirus (Togaviridae family) in Latin America and the Caribbean for which surveillance, diagnostic testing, and genomic data are limited. MAYV infections often are confused with other arboviral illnesses, and no licensed vaccine or specific antiviral treatment is available (*1*). MAYV causes acute and chronic arthritogenic disease (*2*) with fever, rash, and potentially year-long arthralgia (*3*). Typically maintained in the sylvatic cycle by the *Haemagogus janthinomys* mosquito and nonhuman primates (*4*), MAYV frequently spills over to humans (*5*). Experimental evidence suggests urban transmission is possible with *Aedes aegypti* and *A. albopictus* mosquitos (*6*). Increased reports of MAYV infection (*7*,*8*) led us to study the emergence and spread of new Mayaro strains in Peru.

The Study

We collected 1,000 whole blood specimens from patients encountered at 6 health facilities who had acute febrile illness (AFI) during September 2023–October 2024 (Appendix, https://wwwnc.cdc.gov/EID/article/32/12/25-1211-App1.pdf). We sequenced samples by using unbiased, metagenomic next-generation sequencing (NGS) and target-enriched NGS methods (*9*) (Appendix). We initially detected MAYV in 5 persons (5/1,000 [0.5%]) by using teamNGS, yielding 1 complete and 4 partial genome sequences with 13%–78% coverage (Table). BLAST analysis (https://blast.ncbi.nlm.nih.gov) indicated the sequences bore 99% nucleotide identity to a strain isolated from Peru in 2018 (GenBank accession no. KY026196). We inferred a maximum-likelihood phylogeny by using IQ-TREE2 (*10*) (Figure 1), placing all sequences from this study (GenBank accession nos. PZ530724–6) within MAYV genotype D, which splits into 2 monophyletic subclades. One subclade consists mostly of sequences from Peru, including those from patient samples APDC070024 and APDC070034, with a sequence from Colombia at its root. The other subclade shows a similar pattern for patient sample APDC060121. Those results suggest that MAYV diversification in Peru is driven by endemic evolution or repeated introductions, probably from Colombia.

Considering NGS sensitivity limitations and brief viremia periods, we developed a quantitative reverse transcription PCR to detect additional cases of MAYV infection and assess the role of MAYV in AFI in Peru. Our novel, dual-target molecular assay, guided by phylogenetic analysis (Figure 1), targets 2 conserved regions within the nonstructural protein 1 gene, both labeled in the Cy5 channel (Figure 2, panel A; Appendix Figure 1). The assay demonstrated linearity of 10^7–10^1 copies/reaction (R^2 0.99; efficiency 90%) (Figure 2, panel B) and included an internal control for extraction and amplification validation (Figure 2, panel C). Analytical sensitivity (95% CI and probit analysis)

Author affiliations: Abbott Laboratories, Abbott Park, Illinois, USA (L.J. Perez, A. Hodges, K. Forberg, S. Weiss, J. Yamaguchi, M. Mata, M.G. Berg, G.A. Cloherty); Abbott Pandemic Defense Coalition, Abbott Park (L.J. Perez, A. Hodges, K. Forberg, S. Weiss, J. Yamaguchi, M. Mata, M.G. Berg, G.A. Cloherty); Universidad Peruana Cayetano Heredia, Lima, Peru (K. Torres, A. Cruz-Echevarría, D. Cubas, F. Alava, D. Gamboa, J.M. Vinetz); Universidad Peruana Cayetano Heredia, Iquitos, Peru (F. Alava, D. Gamboa, J.M. Vinetz); Yale University, New Haven, Connecticut, USA (J.M. Vinetz)

DOI: https://doi.org/10.3201/eid3212.251211

Table. Clinical manifestations and results of NGS and qRT-PCR for detected cases of Mayaro virus infection, Peru*

Sample ID	Health facility	NGS, % coverage	qRT-PCR, Ct value	Patient age, y/sex	Clinical data
APDC040024	Zungarococha	NA	28.81	16/M	History of fever and chills, malaise, headache, fatigue, loss of appetite, back pain, abdominal pain, joint pain, nonproductive cough, nausea and vomiting, runny nose
APDC070024	San Lorenzo Health Post	64	29.20	19/F	Malaise, abdominal pain
APDC070034	San Lorenzo Health Post	78	29.53	68/M	History of fever and chills, malaise, headache, joint pain.
APDC070108	San Lorenzo Health Post	13	28.61	15/M	History of fever and chills, malaise
APDC010152	Hospital de Apoyo de Iquitos	29	24.67	31/F	History of fever and chills, headache, loss of appetite, joint pain, nausea and vomiting
APDC060121	Bellavista Nanay	100	24.43	17/F	Malaise, loss of appetite, back pain, abdominal pain, joint pain, generalized weakness and paralysis

*Interval of collection for all samples was February–June 2024. Ct, cycle threshold; ID, identification; NA, not available; NGS, next-generation sequencing; qRT-PCR, quantitative reverse transcription PCR.

indicated a limit of detection of ≈4 copies/reaction (Figure 2, panel D). We re-screened 1,000 samples from AFI patients on the Abbott m2000 platform (Abbott Molecular, https://www.molecular.abbott), confirming 5 previously identified positives and detecting 1 additional PCR-positive case missed by NGS (cycle threshold >28), consistent with the detection limits of target-enrichment NGS of log ≈3.0 copies/mL (Table 1).

By using phylodynamic and phylogeographic approaches (*11*), we next sought to understand which

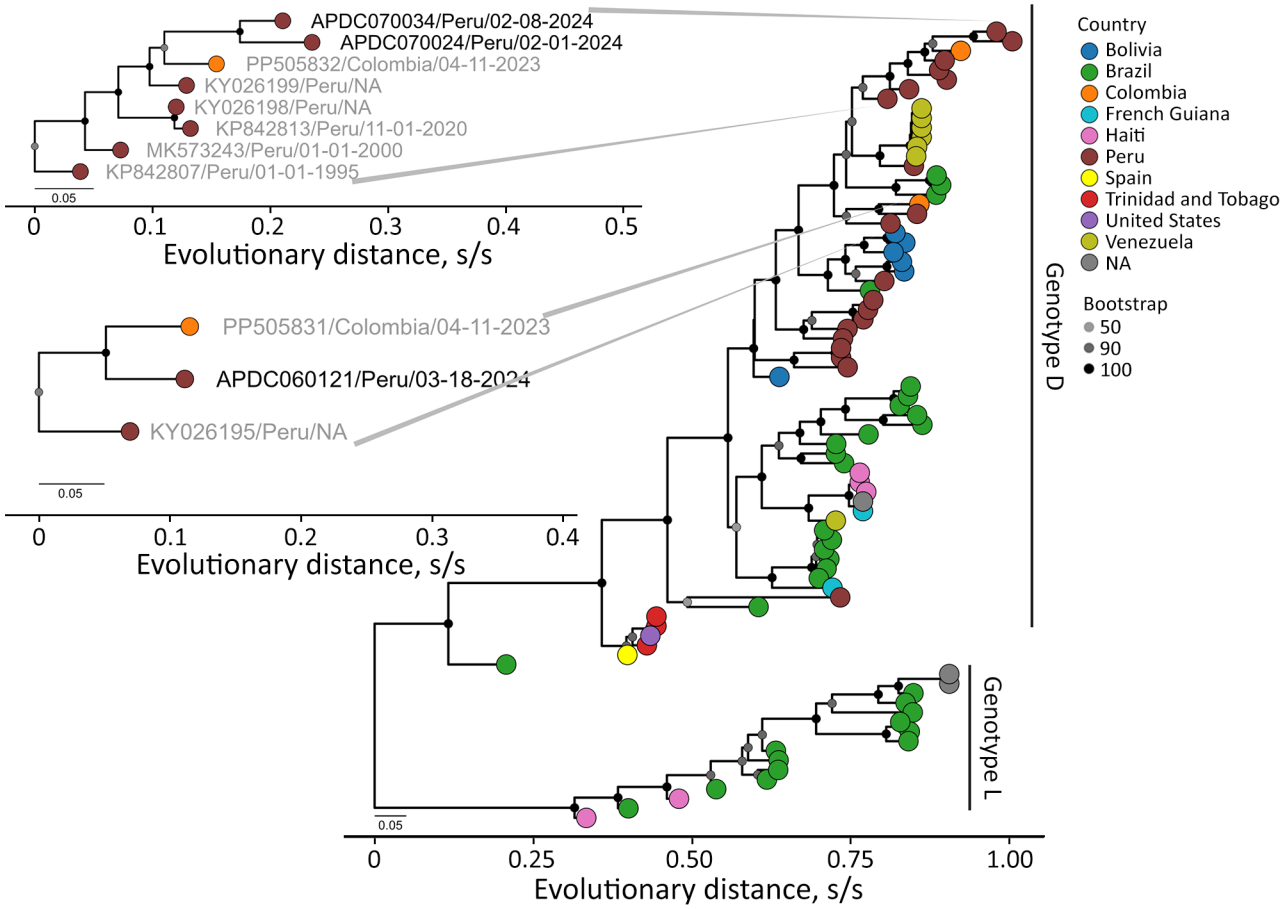


Figure 1. Maximum-likelihood phylogenetic tree of Mayaro virus sequences from Peru compared with global Mayaro virus sequences. All sequences were mapped onto the phylogeny by sampling country and genotype using the ggtreeExtra R package (The R Project for Statistical Computing, https://www.r-project.org). Bootstrap values >80% are shown; scale bars represent nucleotide substitutions per site. Mayaro virus sequences indicate the strains circulating in Peru obtained in this study belong to genotype D and are organized into 2 independent monophyletic clades (left subtrees). NA, not available; s/s, substitution per site.

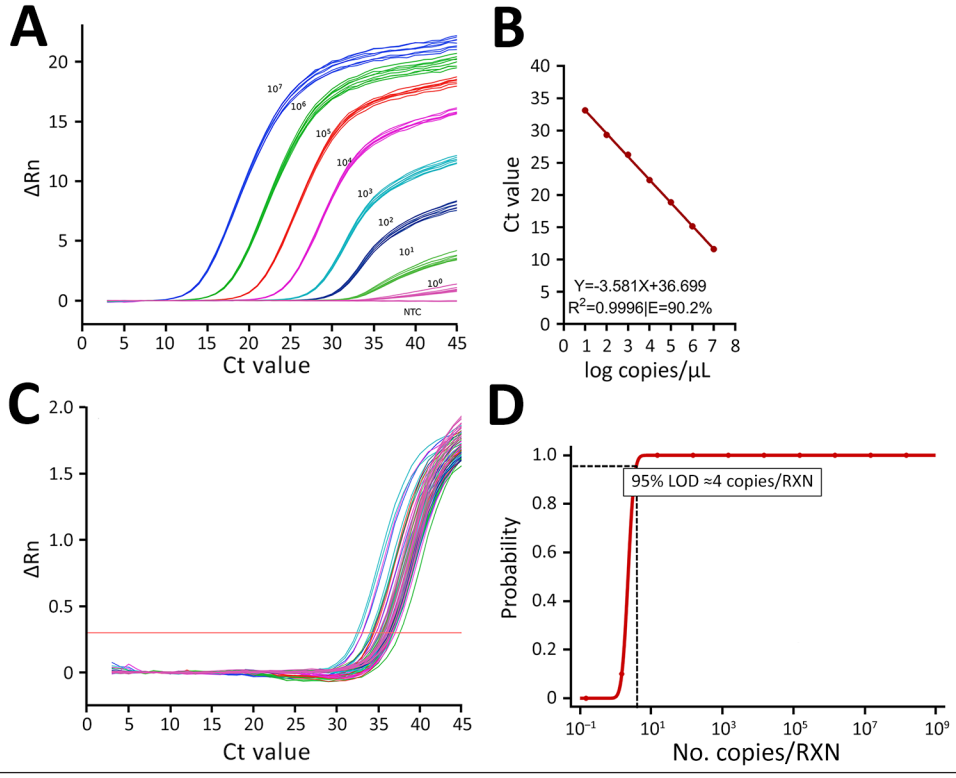


Figure 2. Development and performance evaluation of a novel, dual-target quantitative reverse transcription PCR for Mayaro virus detection. A) Amplification curves from 10-fold serial dilutions of in vitro transcripts from all lineages. B) Assessment of assay linearity. C) Amplification curves for internal control. D) LOD determined through probit analysis of the dilution series. Ct, cycle threshold; LOD, limit of detection; RXN, fluorescence intensity; ΔRn, delta normalized reporter.

MAYV lineages are emergent, where they are originating from, and what is driving their expansion. In our temporal analysis using a Gaussian Markov random field prior (*12*), we traced the emergence of MAYV back to 1886, with an initial split of the 2 main genotypes occurring in 1897 (genotype L) and 1912 (genotype D) (Figure 3, panel A; Appendix Figure 2). The estimated evolutionary rate was 2.6×10^{-4} substitutions/site/year. The analysis also revealed that the cluster containing sequences from Trinidad and Tobago emerged around 1954, coinciding with the first reported case of MAYV infection (*13*), indicating accurate clock calibration in our dataset (Figure 3, panel A). Regarding Peru, we identified multiple clusters

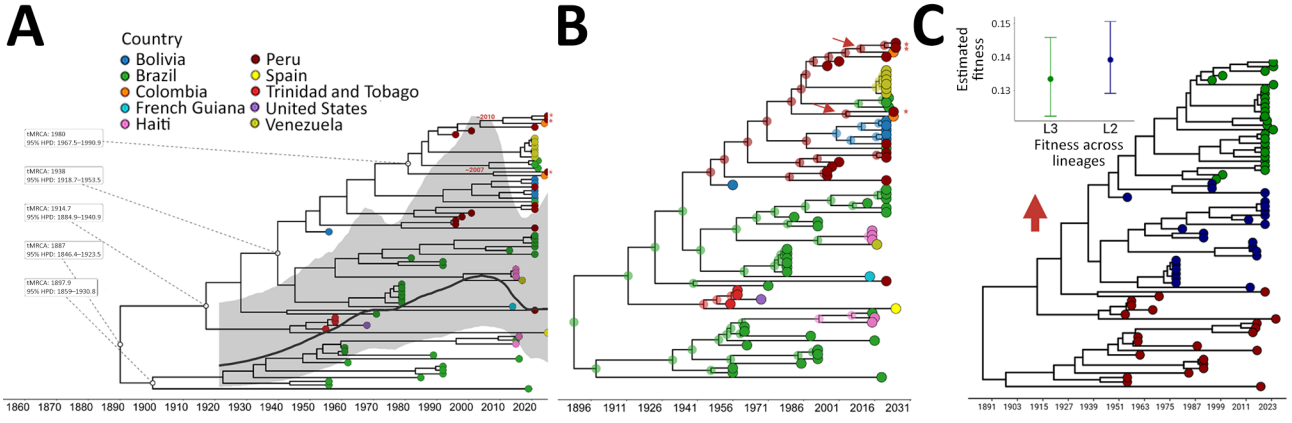


Figure 3. Phylodynamic analysis of emergence, lineage fitness, and explicit landscape dynamics of Mayaro virus lineages. A) Time-scaled maximum clade credibility tree displaying temporal emergence of Mayaro virus strains with tMRCA and 95% HPD intervals for the root and the internal clades of interest. 95% HPD intervals bars for all nodes are shown in Appendix Figure 2 (Appendix, https://wwwnc.cdc.gov/EID/article/32/12/25-1211-App1.pdf). A Bayesian Skyride plot of effective population size over time overlaid to illustrate fluctuations in genetic diversity. Red indicates the tMRCAs of the sequences reported in this study. B) Discrete phylogeographic analysis mapping of Mayaro virus spread across Latin America and the Caribbean; node colors indicate inferred country of origin. Red arrows indicate putative origin for the cluster of the strains reported in this study. C) Time-stamped phylogeny with lineages identified on the basis of index dynamics (inset shows estimation of the relative fitness of each lineage). HPD, highest posterior density; L2, lineage 2; L3, lineage 3; tMRCA, time to most recent common ancestor.

emerging at different timepoints, primarily 40–49 years ago. The cluster containing the APDC070034/Peru/02-08-2024 and APDC070024/Peru/02-01-2024 strains, which grouped with a strain from Columbia (GenBank accession no. PP505832), emerged approximately in 2010, and the ancestor for the APDC060121/Peru/03-18-2024 was circulating around 2007. The Bayesian Skyride reconstruction of the effective population size showed a steady increase in MAYV genetic diversity from around 1925 until 2005. After 2005, a sharp decrease in diversity occurred, reflecting the extinction of several genotype L strains. After that decrease, genetic diversity stabilized, potentially because of the emergence of novel genotype D strains (Figure 3, panel A).

A discrete phylogeographic reconstruction indicated that the root for MAYV emergence originated in Brazil, followed by 2 major dispersal pathways (Figure 3, panel B). The first pathway remained largely confined to Brazil before extending into Haiti (Figure 3, panel B). The second pathway spread from Brazil to Peru and subsequently underwent additional local expansions indicated by the discrete states at internal nodes, which involved Brazil, Trinidad and Tobago, Venezuela, Haiti, and Bolivia. From those foci, multiple exportation events spread into Central America. Those findings highlight the critical role of Brazil and Peru as sources for MAYV lineage evolution and dissemination across South and Central America.

The phylowave tool can recover known circulating lineages and previously unknown ones with considerable fitness advantages (*14*). Moreover, phylowave can agnostically monitor shifts in population composition, even when sequence availability is heavily biased. With this tool, we identified 3 distinct lineages in our dataset (Appendix Figure 3). Two of those (lineages 1 and 2) emerged in the 1930s–1940s, whereas the most recent lineage (lineage 3) arose in the late 1950s. Index value estimations suggest that lineage 1 might have been driven toward extinction, whereas lineages 2 and 3 exhibited cycles of dominance followed by inter-exchanges of lower frequencies. When we mapped the lineages onto our phylogenetic tree, we observed that lineage 1 originated in Brazil, spread to Trinidad and Tobago, and was later found in Louisiana (USA) in a bird sample (*15*) (Figure 3, panel B, C). In contrast, lineage 2 emerged in Brazil and mainly diversified there as well as Haiti and Peru, whereas lineage 3 originated in Peru but subsequently disseminated to countries such as Bolivia, Venezuela, and Brazil, always tracing back to Peru as its ultimate source (Figure 3, panel B, C). A phylowave-based fitness analysis also indicated that lineages 2 and 3 possess comparable genetic fitness, whereas lineage 1 exhibited a lack of fitness, accounting for its apparent path toward extinction (Figure 3, panel C; Appendix Figure 3).

We explored the spatial dispersal patterns of lineages 2 and 3 further by using continuous phylogeographic reconstruction (Appendix Figure 4). For lineage 2, the initial spread occurred between the late 1940s and early 1970s, predominantly confined to Bolivia and Brazil (Appendix Figure 4, panel A). After that phase, viral dispersal intensified in the late 1990s and early 2000s, reaching Venezuela, French Guiana, and Haiti. By contrast, lineage 3 appears to have been introduced into Peru around the late 1970s (Appendix Figure 4, panel B), subsequently spreading from Peru to Bolivia, Venezuela, Brazil, and Colombia from the early 1990s onward. Lineage 2 exhibited a median mean branch dispersal velocity of 10.85 (95% highest posterior density 6.51–21.66), whereas lineage 3 reached a median mean branch dispersal velocity of 40.24 (95% 95% highest posterior density 26.74–290.44). Consequently, lineage 3 appears to have spread ≥4 times faster than lineage 2, possessing a greater capacity to disseminate across South and Central America. This rapid dispersal could explain the elevated number of MAYV infection cases reported in the region and emphasizes the need to strengthen surveillance, particularly in Peru, where this lineage has emerged.

Conclusions

Our findings describe the genetic diversity and dispersal of MAYV across Latin America, highlighting Peru's role in the spread of emerging lineages. The newly developed, dual-target quantitative reverse transcription PCR effectively detects those variants, enabling improved surveillance and control measures against MAYV, especially in regions with increasing case reports and ongoing spillover events.

About the Author

Dr. Perez is a principal research scientist at Abbott Laboratories. His research interests include virus evolution, virus discovery, and molecular diagnostics.

References

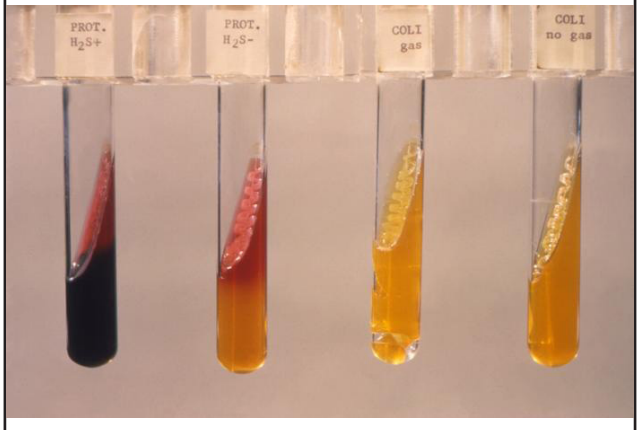
1. Lednicky JA, Ascanio LC, Cardoso S, Chace A, Paniz-Mondolfi AE. Mayaro virus in the Americas: a short review. In: Pujol FH, Paniz-Mondolfi AE, editors. Emerging viruses in Latin America: contemporary virology. Cham, Switzerland: Springer; 2024. p. 139–60.

2. Acosta-Ampudia Y, Monsalve DM, Rodríguez Y, Pacheco Y, Anaya J-M, Ramírez-Santana C. Mayaro: an emerging viral threat? Emerg Microbes Infect. 2018;7:163. https://doi.org/10.1038/s41426-018-0163-5
3. Santiago FW, Halsey ES, Siles C, Vilcarromero S, Guevara C, Silvas JA, et al. Long-term arthralgia after Mayaro virus infection correlates with sustained pro-inflammatory cytokine response. PLoS Negl Trop Dis. 2015;9:e0004104. https://doi.org/10.1371/journal.pntd.0004104
4. Wei LLL, Tom R, Kim YC. Mayaro virus: an emerging alphavirus in the Americas. Viruses. 2024;16:1297. https://doi.org/10.3390/v16081297
5. Celone M, Okech B, Han BA, Forshey BM, Anyamba A, Dunford J, et al. A systematic review and meta-analysis of the potential non-human animal reservoirs and arthropod vectors of the Mayaro virus. PLoS Negl Trop Dis. 2021; 15:e0010016. https://doi.org/10.1371/journal.pntd.0010016
6. Fernández D, Yun R, Zhou J, Parise PL, Mosso-González C, Villasante-Tezanos A, et al. Differential susceptibility of *Aedes aegypti* and *Aedes albopictus* mosquitoes to infection by Mayaro virus strains. Am J Trop Med Hyg. 2023;109:115–22. https://doi.org/10.4269/ajtmh.22-0777
7. Forato J, Meira CA, Claro IM, Amorim MR, de Souza GF, Muraro SP, et al. Molecular epidemiology of Mayaro virus among febrile patients, Roraima State, Brazil, 2018–2021. Emerg Infect Dis. 2024;30:1013–6. https://doi.org/10.3201/eid3005.231406
8. Perez-Restrepo LS, Ciuoderis K, Usuga J, Moreno I, Vargas V, Arévalo-Arbelaez AJ, et al. Mayaro virus as the cause of acute febrile illness in the Colombian Amazon Basin. Front Microbiol. 2024;15:1419637. https://doi.org/10.3389/fmicb.2024.1419637
9. Redman SA, Perez LJ, Forberg K, Francis K, Walker JP, Thompson TK, et al. Dengue virus serotype 3 origins and genetic dynamics, Jamaica. Emerg Infect Dis. 2024;30:2149–54. https://doi.org/10.3201/eid3010.240170
10. Nguyen L-T, 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
11. Pérez LJ, Baele G, Hong SL, Cloherty GA, Berg MG. Ecological changes exacerbating the spread of invasive ticks has driven the dispersal of severe fever with thrombocytopenia syndrome virus throughout Southeast Asia. Mol Biol Evol. 2024; 41:msae173. https://doi.org/10.1093/molbev/msae173
12. Minin VN, Bloomquist EW, Suchard MA. Smooth skyride through a rough skyline: Bayesian coalescent-based inference of population dynamics. Mol Biol Evol. 2008;25:1459–71. https://doi.org/10.1093/molbev/msn090
13. Anderson CR, Downs WG, Wattley GH, Ahin NW, Reese AA. Mayaro virus: a new human disease agent. II. Isolation from blood of patients in Trinidad, B.W.I. Am J Trop Med Hyg. 1957;6:1012–6. https://doi.org/10.4269/ajtmh.1957.6.1012
14. Lefrancq N, Duret L, Bouchez V, Brisse S, Parkhill J, Salje H. Learning the fitness dynamics of pathogens from phylogenies. Nature. 2025;637:683–90. https://doi.org/10.1038/s41586-024-08309-9
15. Calisher CH, Gutiérrez E, Maness KS, Lord RD. Isolation of Mayaro virus from a migrating bird captured in Louisiana in 1967. Bull Pan Am Health Organ. 1974;8:243–8.

Address for correspondence: Michael G. Berg, Abbott Laboratories, 100 Abbott Park Rd, Abbott Park, IL 60064, USA; email: michael.berg@abbott.com

EID Podcast

Reoccurring *Escherichia coli* O157:H7 Strain Linked to Leafy Greens–Associated Outbreaks, 2016–2019



Dr. Jessica Chen, a bioinformatician at CDC in Atlanta, discusses *E. coli* O157:H7 strain linked to leafy greens–associated outbreaks.

Visit our website to listen: https://tools.cdc.gov/medialibrary/index.aspx#/media/id/739173

EMERGING INFECTIOUS DISEASES®