

# NGS Outbreak Investigation

UChicago Center in Paris  
Paris, France  
January 2024

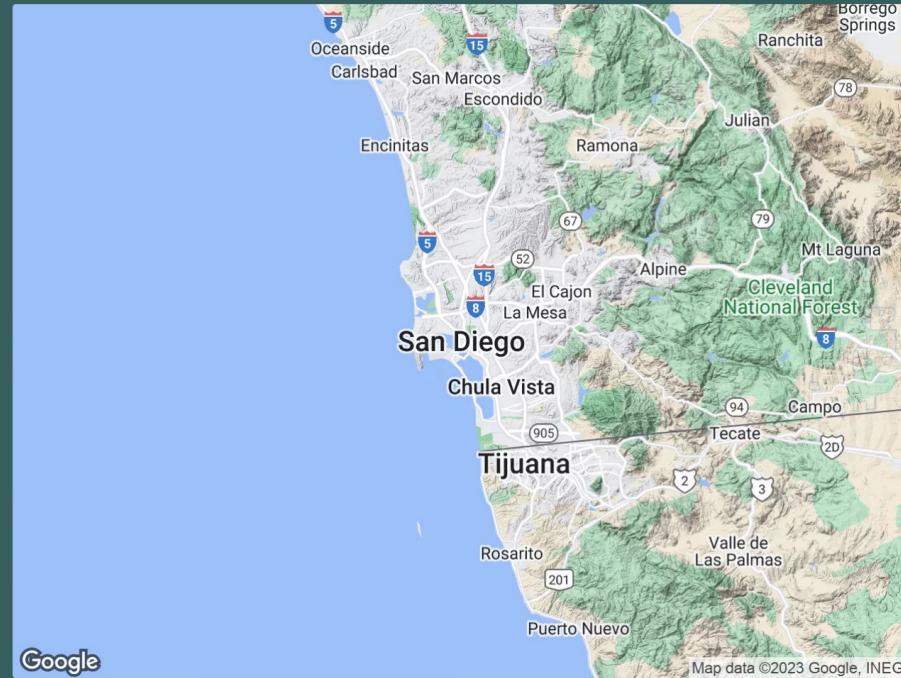
# Good morning!

Disclaimer: The events depicted in this workshop are entirely fictional. Any resemblance to actual persons, living or dead, actual events or outbreaks before or afterwards is purely coincidental.



## ACDC alert – undiagnosed febrile illness

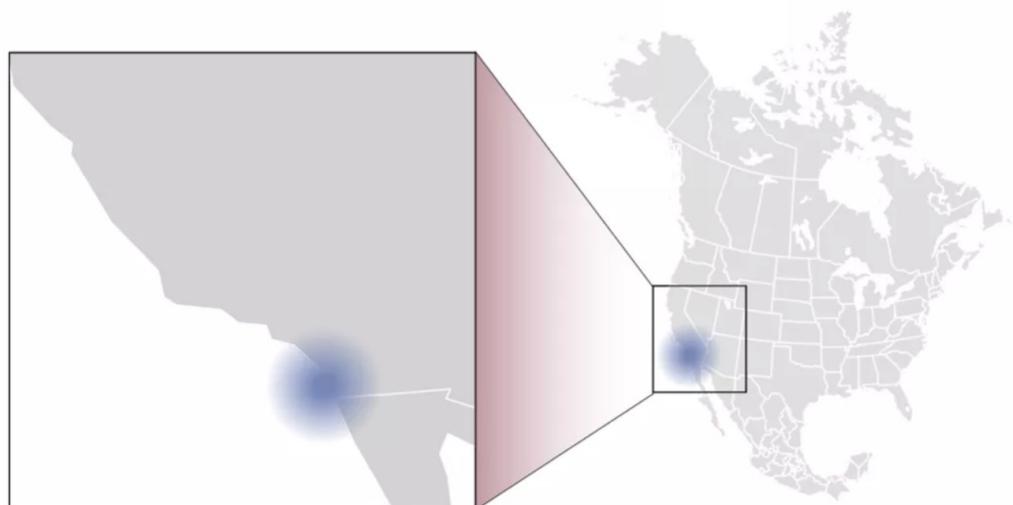
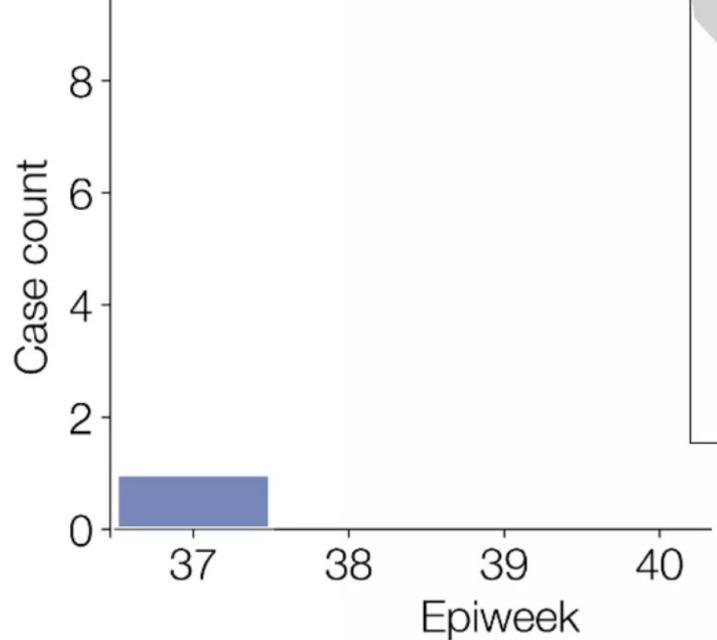
2022-09-12: A 30 year old male in Southern California presented to the emergency department complaining of fever, tiredness, aches. On examination he has a temperature of 38.3C and is jaundiced. His ALP is 220U/ml with platelets of 10. Over the course of the day he becomes progressively more unwell and begins bleeding profusely. Viral screen including hepatitis A-E is negative.



What is your diagnosis?

2022-09-12 | Situation Report #1: Undiagnosed febrile illness, Southern California, 2022

Author: Donald McConnald PhD, ACDC outbreak investigation director.

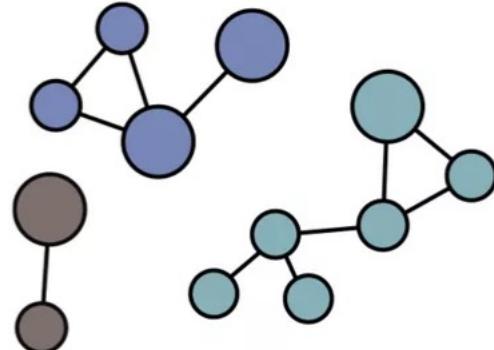


# What should we try?

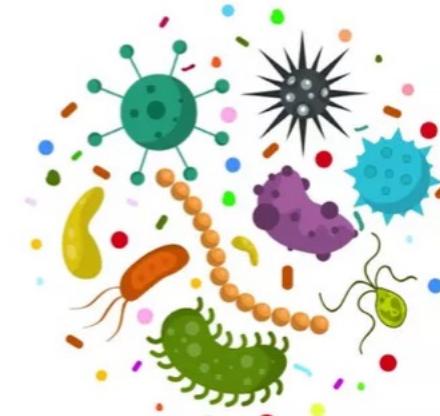
Course of action:



Targeted fever panel

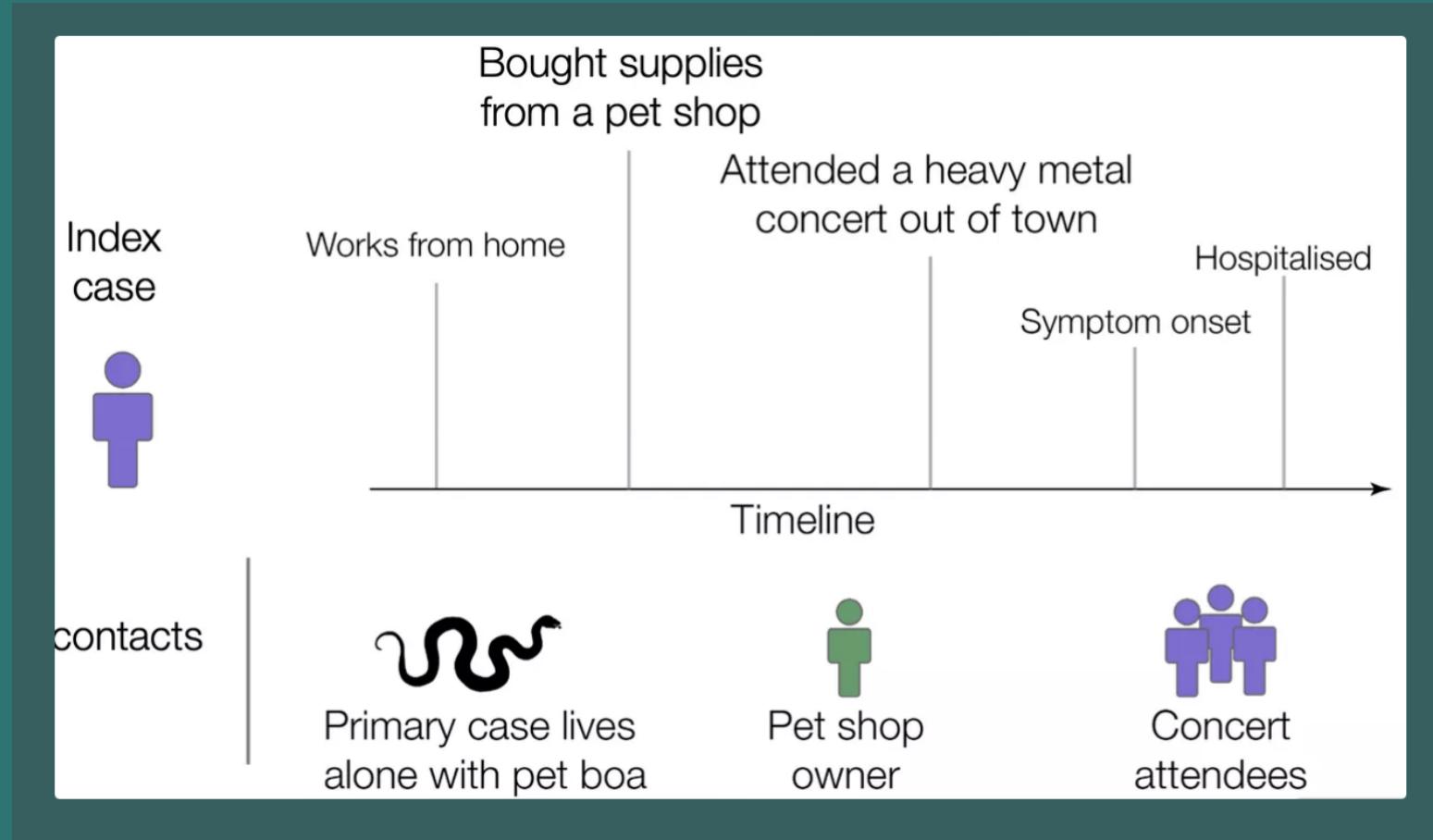


Epidemiological investigation/ questions



Metagenomic sequencing

# Enhanced epidemiology investigation



# Global fever panel

Global Fever Panel			
Sample ID: CA-Vir-0001 Operator: Bill	Run date: 2022-09-21 Run status: complete Controls: passed	Instrument: VirDiag-5000-pro Protocol: Whole blood	
Result: all negative			
Results summary			
<b>Detected</b> None	<b>Not-detected</b> Chikungunya virus Crimean-Congo hemorrhagic fever virus Dengue virus Ebola virus Lassa virus Marburg virus West Nile virus Yellow fever virus Zika virus Bacillus anthracis Francisella tularensis Leptospira spp. Salmonella enterica serovar Paratyphi Salmonella enterica serovar Typhi Yersinia pestis Leishmania donovani complex Plasmodium spp. Plasmodium falciparum Plasmodium vivax/ovale		

## Metagenomic Sequencing of Index Case

Total DNA and RNA extracted from blood of the primary case was sequenced using Illumina technology with paired 150bp reads at a willing academic laboratory that routinely sequences microbial genomes and metagenomes. The sequencing generated around 1M reads in total. There was no target enrichment for specific pathogens. Bioinformatics analysis was performed with Kraken2 of reads searched against Ben Langmead's "Standard" microbial taxonomic database.

[https://artic-network.github.io/artic-live/gc/index\\_case\\_title.html](https://artic-network.github.io/artic-live/gc/index_case_title.html)

What plausible pathogenic agents did you find?

**2022-09-20 | Situation Report #2: Undiagnosed febrile illness, Southern California, 2022**

**Author: Donald McConnald PhD, ACDC outbreak investigation director.**

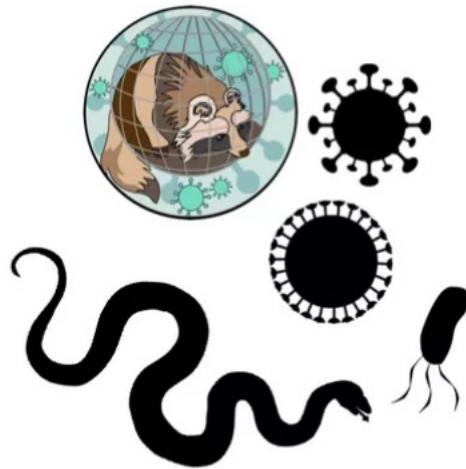
## **Executive Summary**

Officials at the ACDC, along with the local public health department, have identified suspected cases in the San Diego metropolitan area with similar clinical symptoms. Contact tracing efforts are currently underway and samples are being taken. At least 2 report attendance at the same concert as the index case.

# What do we sequence next?



Wastewater sample  
metropolitan area

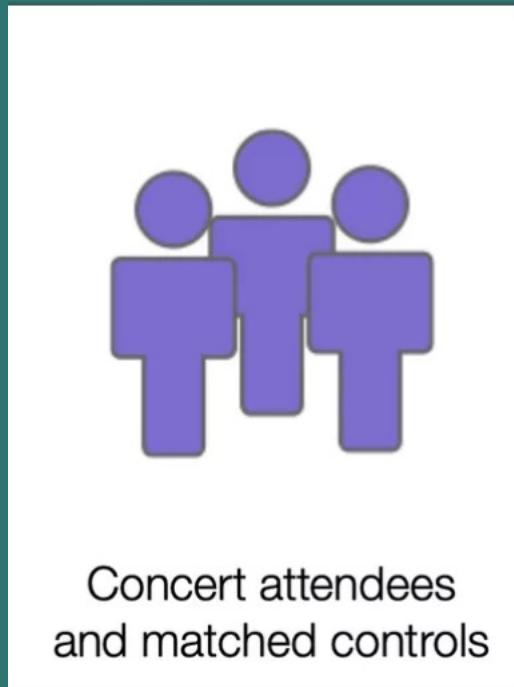


Environmental  
sampling in pet shop



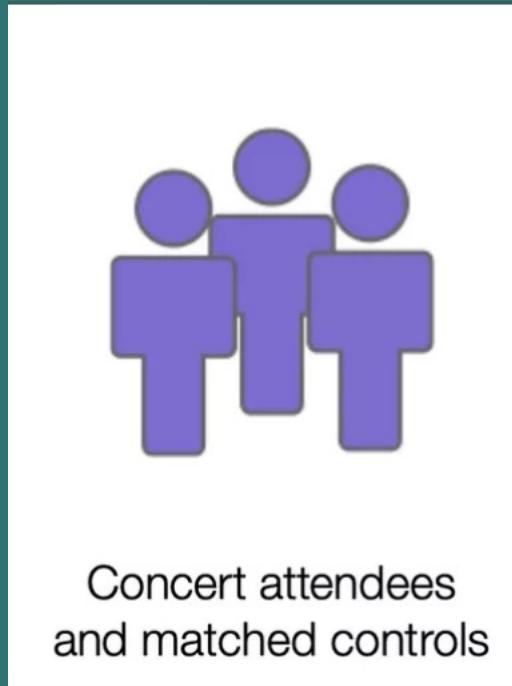
Concert attendees  
and matched controls

# Heavy Metal Associated Cases



[https://artic-network.github.io/artic-live/gc/case\\_2\\_title.html](https://artic-network.github.io/artic-live/gc/case_2_title.html)  
[https://artic-network.github.io/artic-live/gc/case\\_3\\_title.html](https://artic-network.github.io/artic-live/gc/case_3_title.html)  
[https://artic-network.github.io/artic-live/gc/case\\_4\\_title.html](https://artic-network.github.io/artic-live/gc/case_4_title.html)

# Heavy Metal Associated Controls

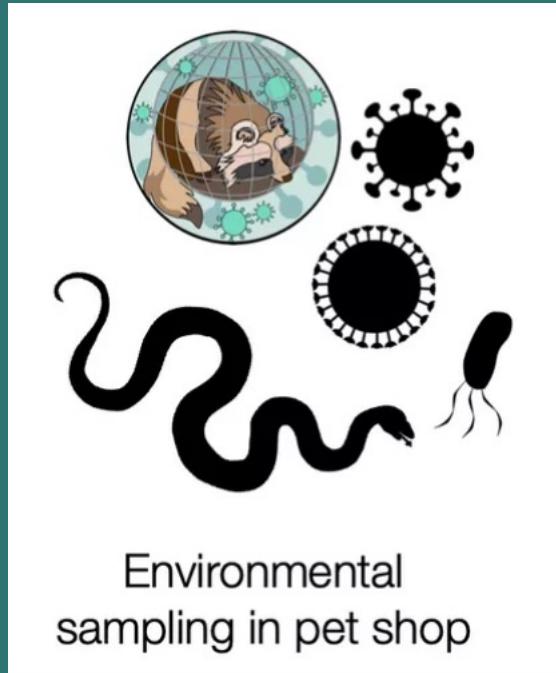


[https://artic-network.github.io/artic-live/gc/control\\_1\\_title.html](https://artic-network.github.io/artic-live/gc/control_1_title.html)

[https://artic-network.github.io/artic-live/gc/control\\_2\\_title.html](https://artic-network.github.io/artic-live/gc/control_2_title.html)

[https://artic-network.github.io/artic-live/gc/control\\_3\\_title.html](https://artic-network.github.io/artic-live/gc/control_3_title.html)

# Pet Shop Environmental Sampling



[https://artic-network.github.io/artic-live/gc/pet\\_shop\\_env\\_title.html](https://artic-network.github.io/artic-live/gc/pet_shop_env_title.html)

# Wastewater Sampling



[https://artic-network.github.io/artic-live/gc/wastewater\\_seq\\_title.html](https://artic-network.github.io/artic-live/gc/wastewater_seq_title.html)

What interventions are appropriate?

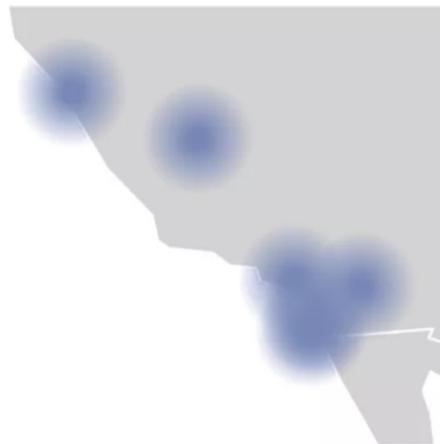
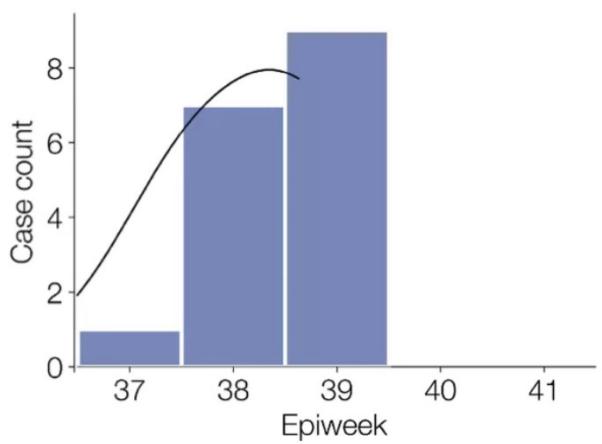
What pathogens are you ruling out and why?

What is the likely causative agent?

**To: ProMED-mail**

**Subject: Cases of fever and hepatitis associated with a heavy metal rock concert spread to additional locations**

Increasing cases of individuals presenting with acute fever and hepatitis reported, which have been linked to attendance of a heavy metal rock festival, 'Ozzmium', in Peaky, California. Cases are present in a number of urban centres, including San Francisco, Fresno and San Diego.



2022-09-25 | Situation Report #3: Undiagnosed febrile illness, Southern California, 2022

Author: Donald McConnald PhD, ACDC outbreak investigation director.



## Executive Summary

Given the association with snakes and metal music, public health officials are concerned about the upcoming Repticon™, as well as upcoming live amplified music events.



## Assembled Genome Segments

Segment S - [https://artic-network.github.io/artic-live/gc/segment\\_S.fasta.txt](https://artic-network.github.io/artic-live/gc/segment_S.fasta.txt)

Segment L - [https://artic-network.github.io/artic-live/gc/segment\\_L.fasta.txt](https://artic-network.github.io/artic-live/gc/segment_L.fasta.txt)

Mammarenavirus draft genome

>segmentL

GCGCAGCGTGGAGCCTAGGCTTACTTGACATT...

>segments

CGCGCGGTGGATCCTAGGGTTTACTTGAAC...

[https://artic-network.github.io/artic-live/gc/segment\\_S.fasta.txt](https://artic-network.github.io/artic-live/gc/segment_S.fasta.txt)

[https://artic-network.github.io/artic-live/gc/segment\\_L.fasta.txt](https://artic-network.github.io/artic-live/gc/segment_L.fasta.txt)

# NCBI Blast

Paste your sequence in the  
Query Sequence box

The screenshot shows the NCBI Standard Nucleotide BLAST search page. At the top, there's a navigation bar with tabs for 'blastn', 'blastp', 'blastx', 'tblastn', and 'tblastx'. Below the tabs, there's a search form with fields for 'Enter Query Sequence' (with a placeholder 'Enter accession number(s), gi(s), or FASTA sequence(s)'), 'From' and 'To' range inputs, and a file upload section. There are also fields for 'Job Title' and 'Align two or more sequences'. Under 'Database', there are radio buttons for 'Standard databases (nr etc.)', 'rRNA/ITS databases', 'Genomic + transcript databases', and 'Betacoronavirus'. A link 'Try experimental taxonomic nt databases' leads to a sub-page. The 'Organism' section includes a dropdown for 'Nucleotide collection (nr/nt)', a search bar for 'Enter organism name or id—completions will be suggested', and checkboxes for 'Exclude' (models, uncultured/environmental sample sequences, sequences from type material). The 'Program Selection' section has radio buttons for 'Highly similar sequences (megablast)', 'Most similar sequences (blastnplus megablast)', and 'Somewhat similar sequences (blastx)'. A link 'Choose a BLAST algorithm' is also present.

Nucleotide BLAST: Search  
nucleotide databases using a  
nucleotide query

An official website of the United States govern...  
blast.ncbi.nlm.nih.gov

What is this virus?

# Segment L BLAST results

Organism	Blast Name	Score	Number of Hits	Description
root			110	
· Arenaviridae	viruses		109	
· · Mammarenavirus	viruses		106	
· · · Bear Canyon mammarenavirus	viruses	6715	4	Bear Canyon mammarenavirus hits
· · · Whitewater Arroyo mammarenavirus	viruses	2472	12	Whitewater Arroyo mammarenavirus hits
· · · Tamiami mammarenavirus	viruses	1027	6	Tamiami mammarenavirus hits
· · · Flexal mammarenavirus	viruses	541	1	Flexal mammarenavirus hits
· · · Pirital mammarenavirus	viruses	403	2	Pirital mammarenavirus hits
· · · Alipahuayo mammarenavirus	viruses	296	1	Alipahuayo mammarenavirus hits
· · · Pichinde virus	viruses	246	6	Pichinde virus hits
· · · Parana virus	viruses	233	1	Parana virus hits
· · · Catarina virus	viruses	220	4	Catarina virus hits
· · · Skinner Tank virus	viruses	201	1	Skinner Tank virus hits
· · · Real de Catorce virus	viruses	194	1	Real de Catorce virus hits
· · · Big brushy tank virus	viruses	174	4	Big brushy tank virus hits
· · · Tonto creek virus	viruses	145	4	Tonto creek virus hits
· · · Latino mammarenavirus	viruses	143	1	Latino mammarenavirus hits
· · · Wufeng Niviventer niviventer mammarenavirus 1	viruses	104	1	Wufeng Niviventer niviventer mammarenavirus hits
· · · Wenzhou mammarenavirus	viruses	98.7	21	Wenzhou mammarenavirus hits
· · · Machupo mammarenavirus	viruses	98.7	9	Machupo mammarenavirus hits
· · · Lassa mammarenavirus	viruses	98.7	19	Lassa mammarenavirus hits
· · · Amapari virus	viruses	96.0	2	Amapari virus hits
· · · Xingyi virus	viruses	93.3	2	Xingyi virus hits
· · · Wenzhou Rattus losea mammarenavirus 1	viruses	92.4	1	Wenzhou Rattus losea mammarenavirus hits
· · · Rat mammarenavirus	viruses	91.5	2	Rat mammarenavirus hits
· · · Wenzhou Rattus tanezumi mammarenavirus 1	viruses	76.1	1	Wenzhou Rattus tanezumi mammarenavirus hits
· · Black Mesa virus	viruses	287	1	Black Mesa virus hits
· · Orogrande virus	viruses	217	1	Orogrande virus hits
· · Patawa virus	viruses	141	1	Patawa virus hits
· · Cloning vector pSF_lenti LASV Lineage IV L-gene	other sequences	88.7	1	Cloning vector pSF_lenti LASV Lineage IV L-gene hits

# Segment S BLAST results

Organism	Blast Name	Score	Number of Hits	Description
<a href="#">Arenaviridae</a>	<a href="#">viruses</a>		<u>120</u>	
· <a href="#">Mammarenavirus</a>	<a href="#">viruses</a>		<u>114</u>	
· · <a href="#">Whitewater Arroyo mammarenavirus</a>	<a href="#">viruses</a>	3269	<u>26</u>	<a href="#">Whitewater Arroyo mammarenavirus hits</a>
· · <a href="#">North American arenavirus</a>	<a href="#">viruses</a>	1691	<u>18</u>	<a href="#">North American arenavirus hits</a>
· · <a href="#">Arenavirus AV 96010025</a>	<a href="#">viruses</a>	1427	<u>1</u>	<a href="#">Arenavirus AV 96010025 hits</a>
· · <a href="#">Arenavirus H0380005</a>	<a href="#">viruses</a>	1210	<u>1</u>	<a href="#">Arenavirus H0380005 hits</a>
· · <a href="#">Bear Canyon mammarenavirus</a>	<a href="#">viruses</a>	1095	<u>18</u>	<a href="#">Bear Canyon mammarenavirus hits</a>
· · <a href="#">Tonto creek virus</a>	<a href="#">viruses</a>	1054	<u>3</u>	<a href="#">Tonto creek virus hits</a>
· · <a href="#">Big brushy tank virus</a>	<a href="#">viruses</a>	1049	<u>6</u>	<a href="#">Big brushy tank virus hits</a>
· · <a href="#">Catarina virus</a>	<a href="#">viruses</a>	1005	<u>38</u>	<a href="#">Catarina virus hits</a>
· · <a href="#">Skinner Tank virus</a>	<a href="#">viruses</a>	899	<u>1</u>	<a href="#">Skinner Tank virus hits</a>
· · <a href="#">Tamiami mammarenavirus</a>	<a href="#">viruses</a>	527	<u>2</u>	<a href="#">Tamiami mammarenavirus hits</a>
· <a href="#">Arenavirus H0030026</a>	<a href="#">viruses</a>	967	<u>1</u>	<a href="#">Arenavirus H0030026 hits</a>
· <a href="#">Black Mesa virus</a>	<a href="#">viruses</a>	891	<u>4</u>	<a href="#">Black Mesa virus hits</a>
· <a href="#">Arenavirus H0030028</a>	<a href="#">viruses</a>	311	<u>1</u>	<a href="#">Arenavirus H0030028 hits</a>

What should the virus be named?

# Heavy Metal Fever Virus (HMFV)

## Situation Report - 2



Data as reported by 20th September 2022

### HIGHLIGHTS

Following the WHO best practices for naming of new human infectious diseases, which were developed in consultation and collaboration with the World Organisation for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO), WHO and ICTV have formed a working group to name the virus.

Twitter has informally named it Heavy Metal Fever Virus (#HMFV).



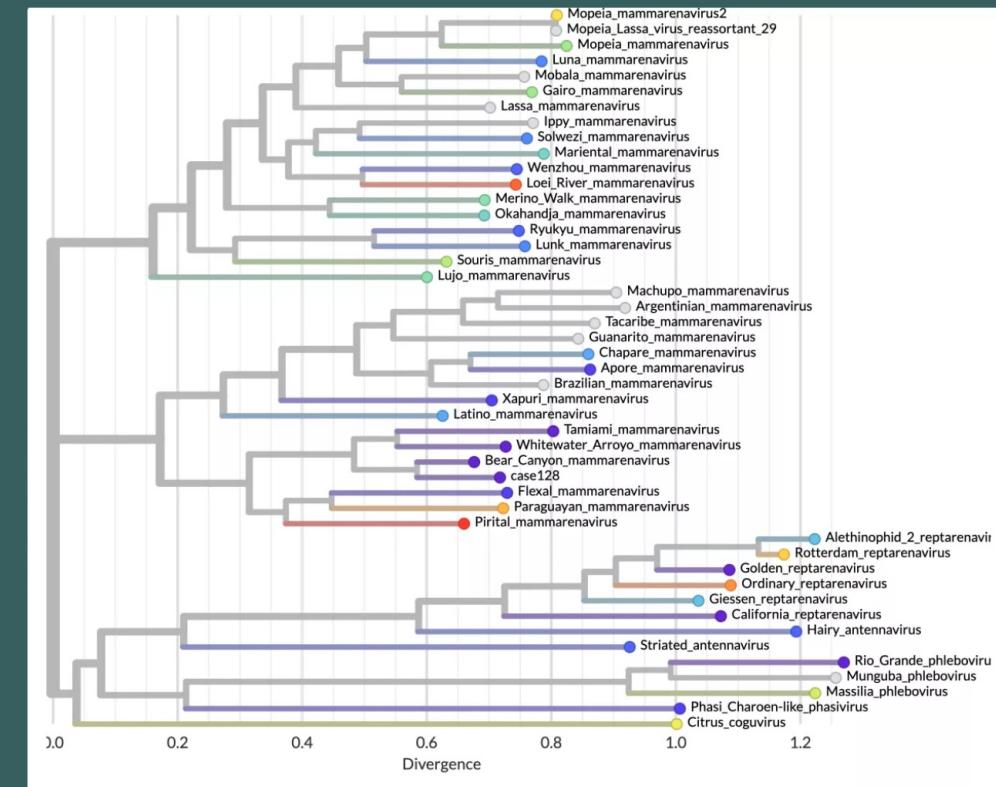
# Evolutionary History Virus Phylogeny

Segment L:

<https://nextstrain.org/community/emmahodcroft/GC/arenavirus/L>

Segment S:

<https://nextstrain.org/community/emmahodcroft/GC/arenavirus/S>

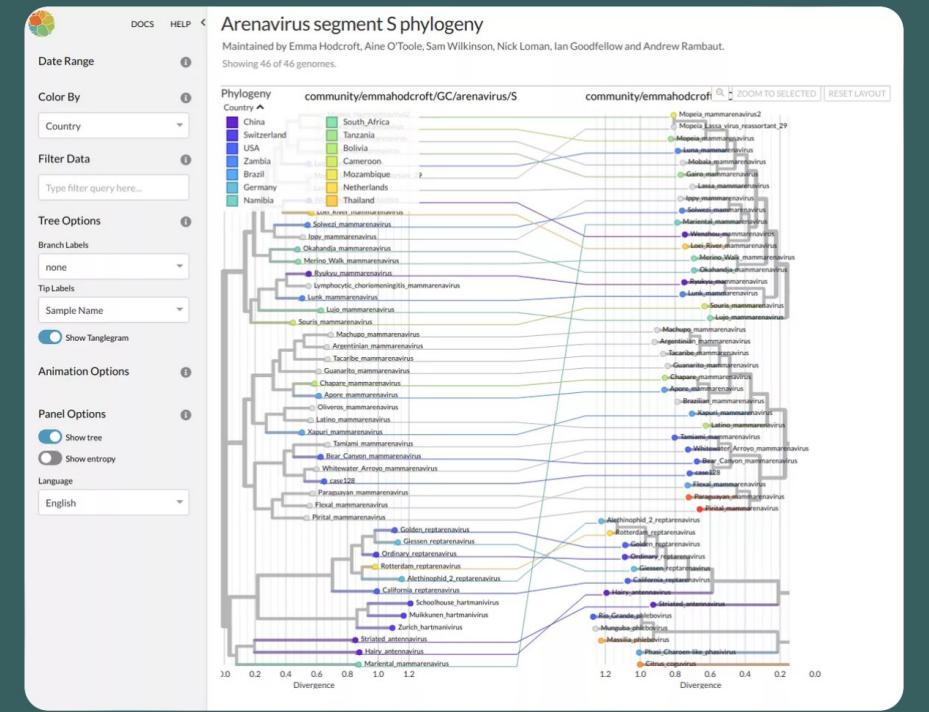


Segment L: <https://nextstrain.org/community/emmahodcroft/GC/arenavirus/L>

Segment S: <https://nextstrain.org/community/emmahodcroft/GC/arenavirus/S>

# Tanglegram of Segment S/L Phylogenies

<https://nextstrain.org/community/emmahodcroft/GC/arenavirus/S:community/emmahodcroft/GC/arena-virus/L>



## auspice

nextstrain.org

<https://nextstrain.org/community/emmahodcroft/GC/arenavirus/S:community/emmahodcroft/GC/arenavirus/L>

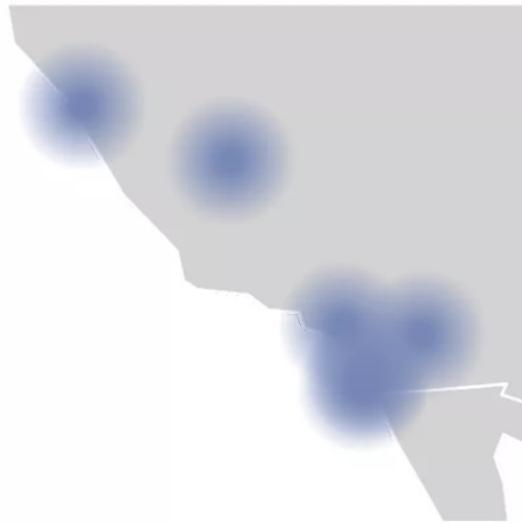
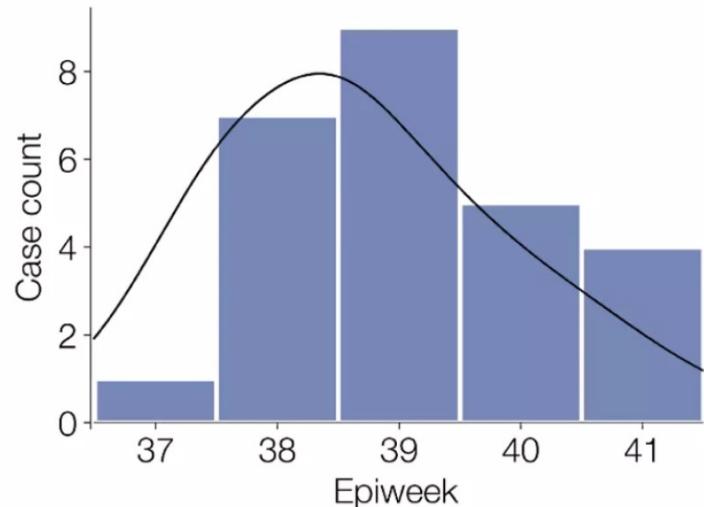
Advise policymakers on what this can tell us  
about the novel virus

**To: ProMED-mail**

**Subject: Further identification of heavy-metal related hemorrhagic fever**

ACDC health officer Donald McConnald, PhD. has issued a statement regarding the HMFV outbreak. New cases have come to light that are secondary household cases, suggesting that human to human transmission is possible.

He advised that "the ACDC are monitoring the situation closely" and that "heavy metal fans across Southern California should avoid contact with potential cases by not attending further metal concerts and by listening to some other music for once".





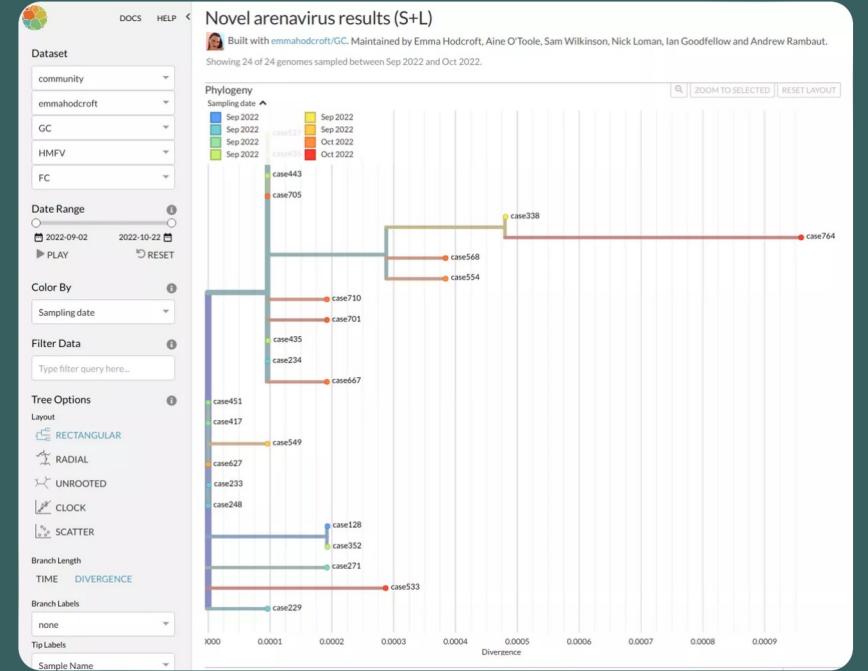
**To: ProMED-mail**

**Subject: No mammarenavirus detected in pet shop mice**

Sampling of frozen mice in *Le Marché Humide* pet shop, linked to the index case, has not shown up any evidence of the HMFV mammarenavirus. Attendees up the upcoming Repticon breathe a sigh of relief.

# Metal cluster sequencing

[https://nextstrain.org/community/  
emmahodcroft/GC/HMFV/FC](https://nextstrain.org/community/emmahodcroft/GC/HMFV/FC)



auspiceweb

[nextstrain.org](https://nextstrain.org)

[https://nextstrain.org/community/  
emmahodcroft/GC/HMFV/FC](https://nextstrain.org/community/emmahodcroft/GC/HMFV/FC)

**ACDC Alert : Two additional cases identified unlinked to a metal music event**

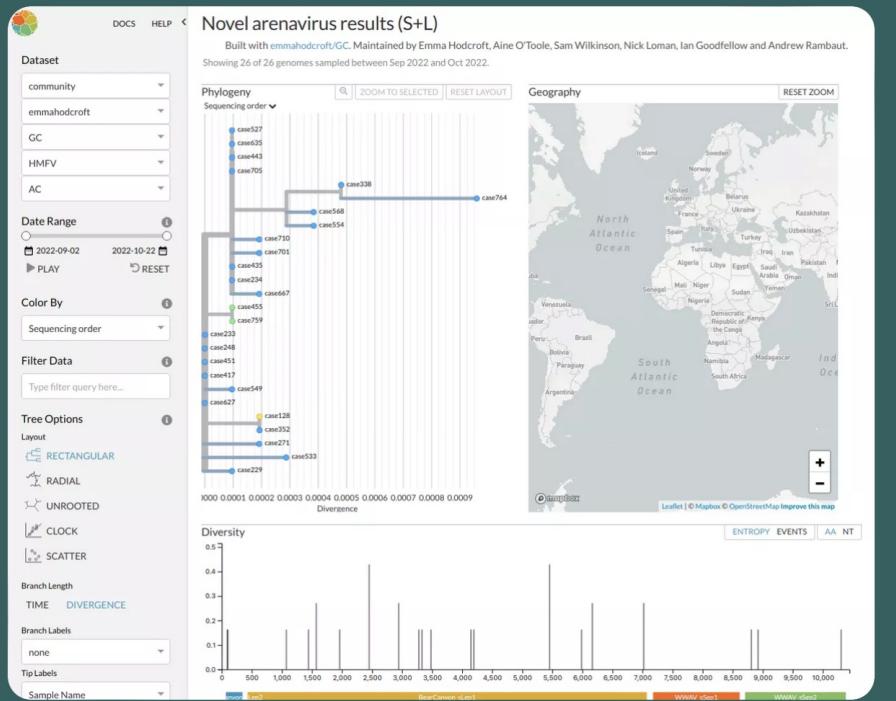
**Author: Donald McConnald PhD, ACDC outbreak investigation director.**

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Two additional cases have been identified with no epidemiological link to the rock concert. Both individuals have been admitted to hospital following a camping trip to Peaky, California.



# Phylogenetic update 2

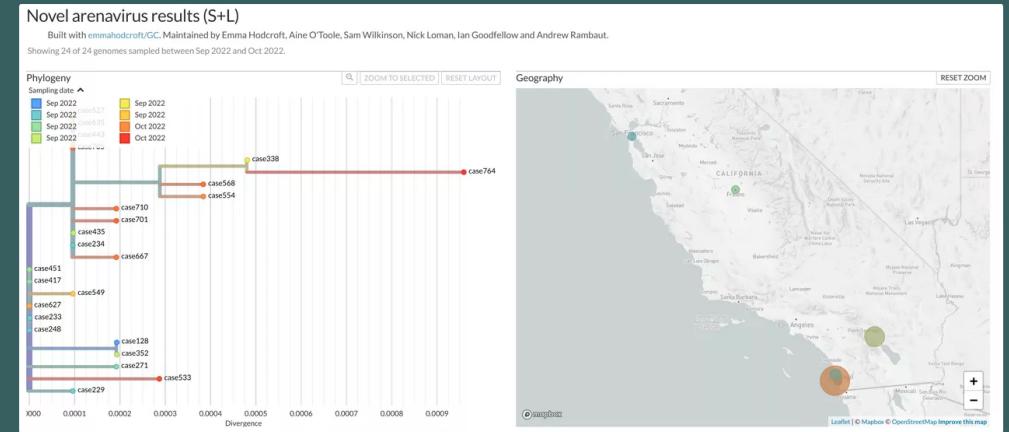


auspiceweb

[nextstrain.org](https://nextstrain.org)

# Phylogenetic analysis of rock concert cases

[https://nextstrain.org/community/  
emmahodcroft/GC/HMFV/FC?  
p=grid](https://nextstrain.org/community/emmahodcroft/GC/HMFV/FC?p=grid)



<https://nextstrain.org/community/emmahodcroft/GC/HMFV/FC?p=grid>

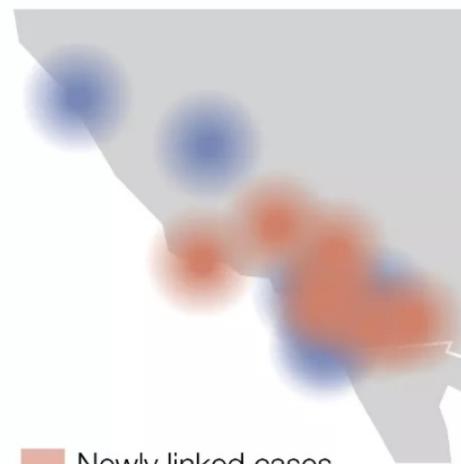
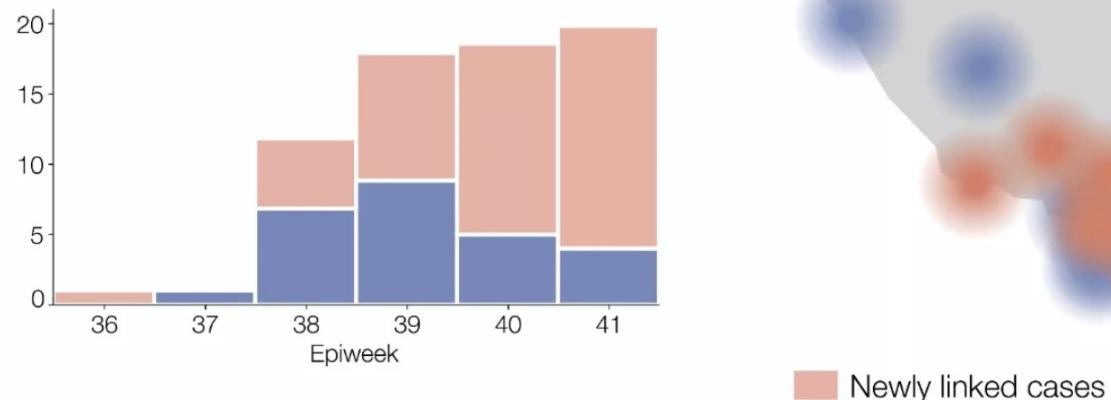
What can this tell us about the outbreak  
and when it may have started?

## Technical Report: Additional cases with shared clinical symptoms identified in rural Southern California

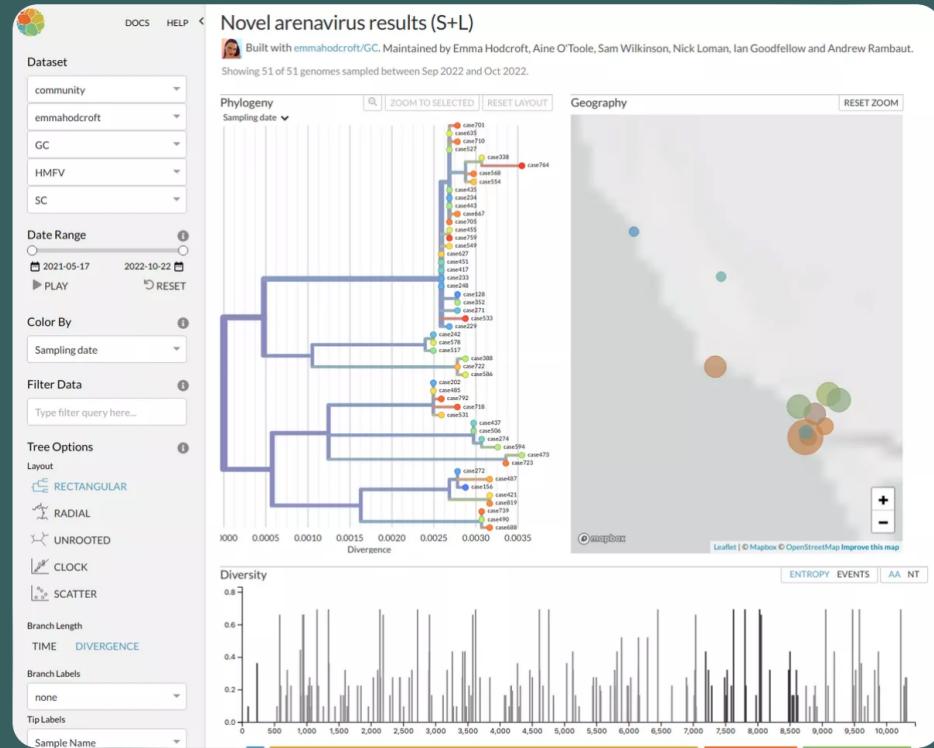
American Center for Disease Combatting

### Executive Summary

Public health experts at the ACDC, along with local clinicians, have identified new cases with shared clinical symptoms to HMFV. Cases have no epidemiological link to the heavy-metal associated superspreading event and are scattered throughout Southern California (Badwater Basin, Bummerville, Gas Point and Death Valley). Patients have not reported any particular association with either death metal or reptiles. Of suspected cases, 70% have recent history of hiking and/or camping in local trails within a week prior to symptom onset. Confirmatory sequencing was carried out in a subset of these suspected cases of HMFV.



# Final Nextstrain Phylogeny

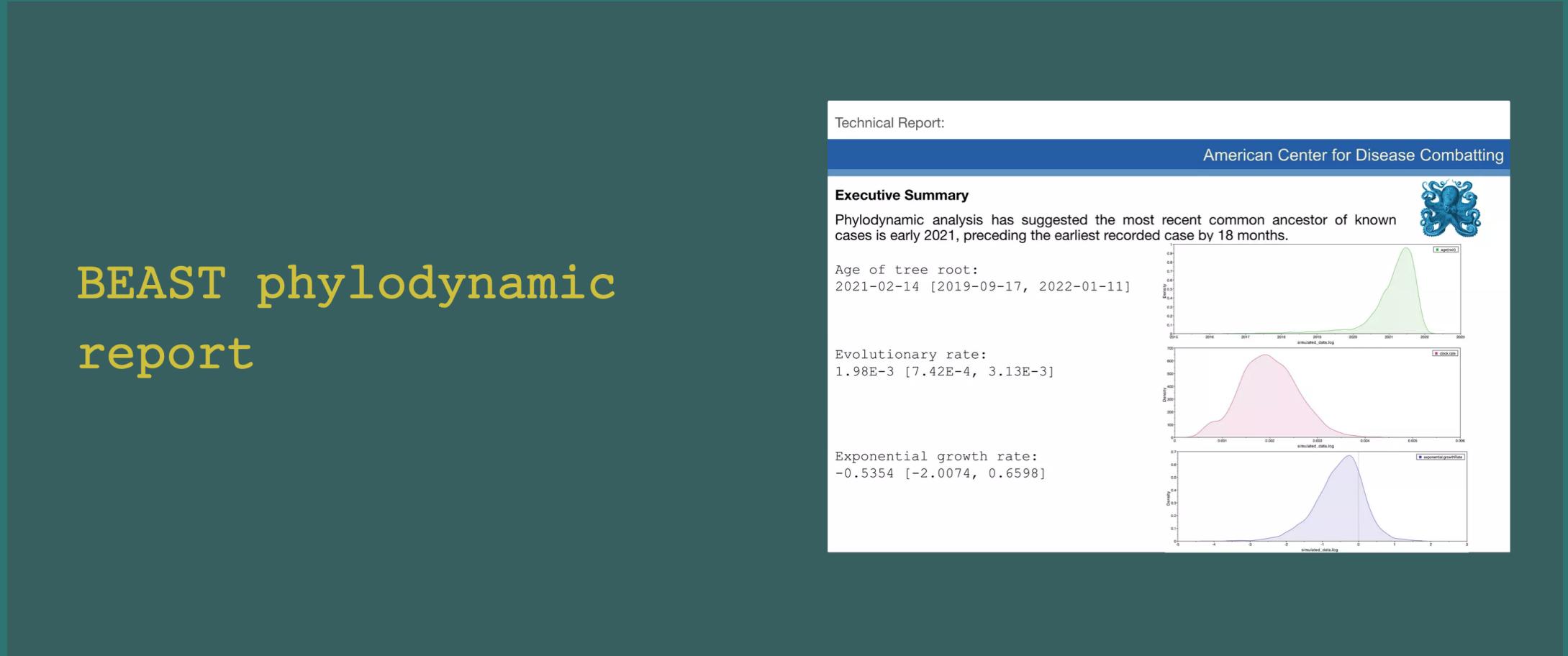


auspicie

[nextstrain.org](https://nextstrain.org)

<https://nextstrain.org/community/emmahodcroft/GC/HMFV/SC?p=grid>

# BEAST phylodynamic report



Does this change what we think about  
the outbreak?

How do we stop this outbreak from  
becoming a pandemic?

