

NGS Outbreak Investigation

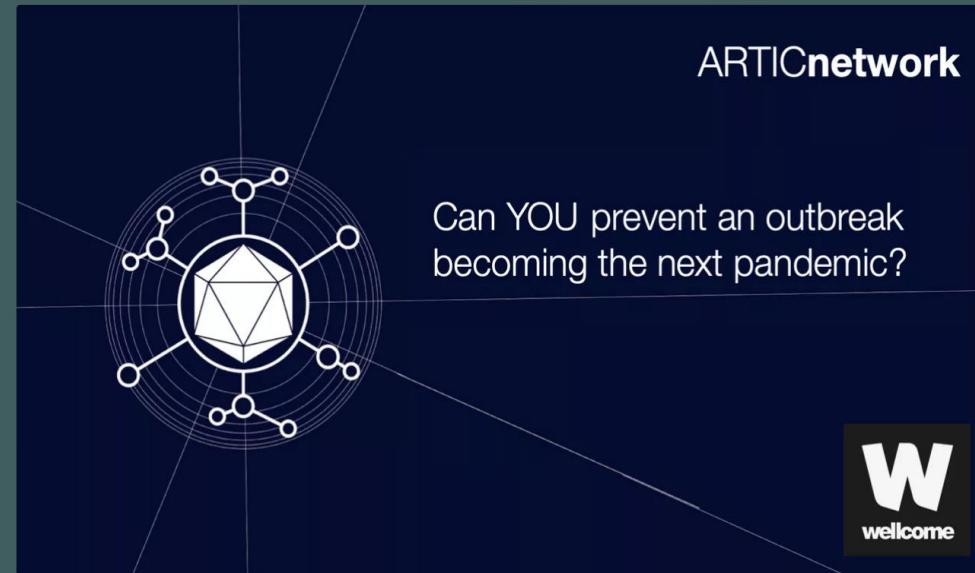
UChicago Center in Paris

Paris, France

January 2025

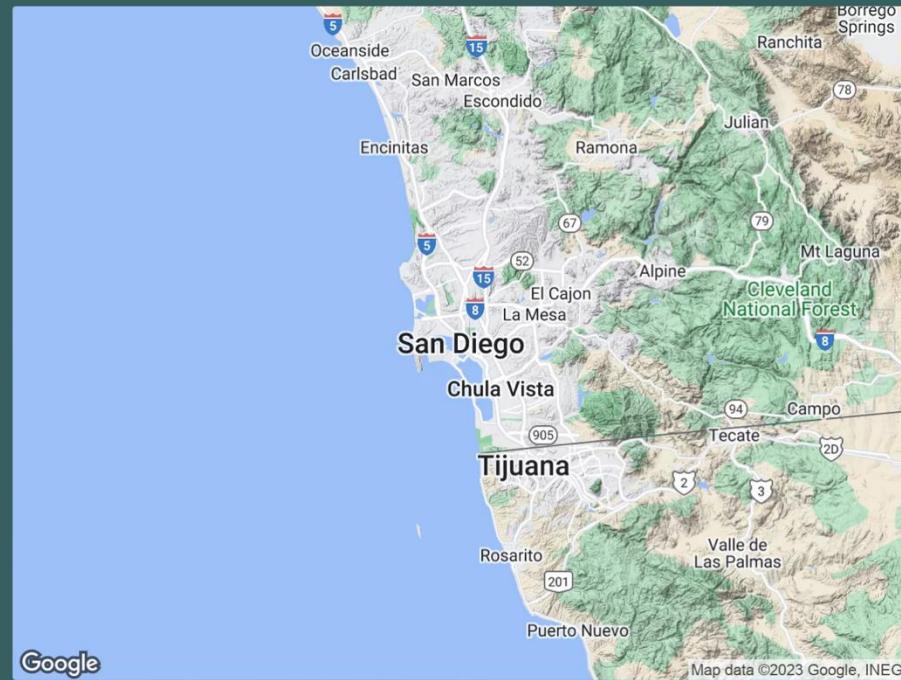
Good morning!

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.



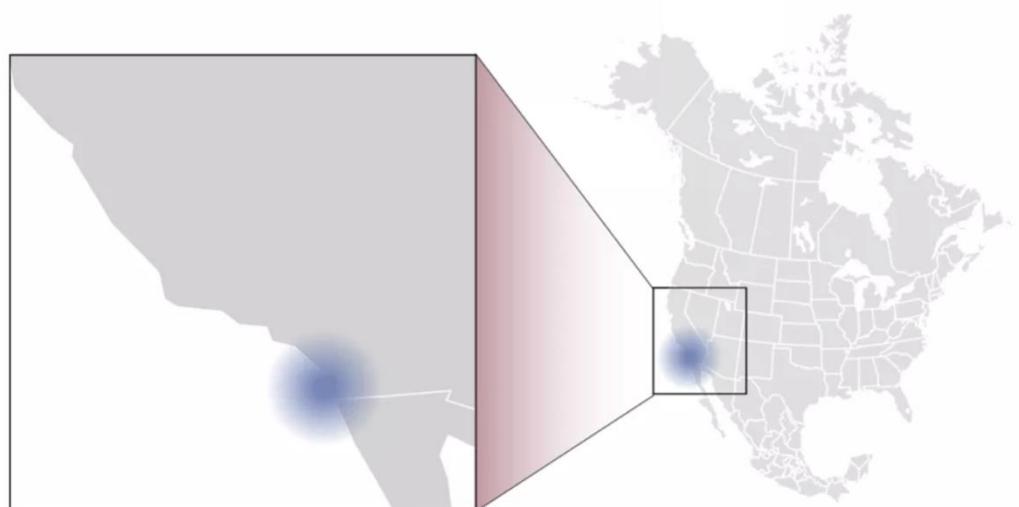
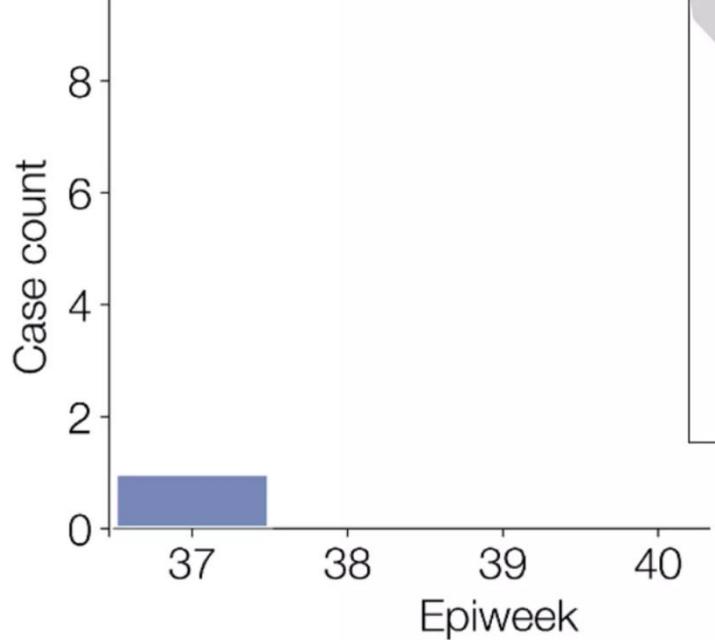
Google

Map data ©2023 Google, INEGI

What is your diagnosis?

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

Author: Donald McConnald PhD, ACDC outbreak investigation director.

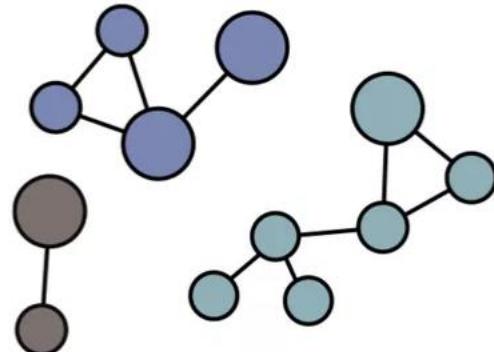


What should we try?

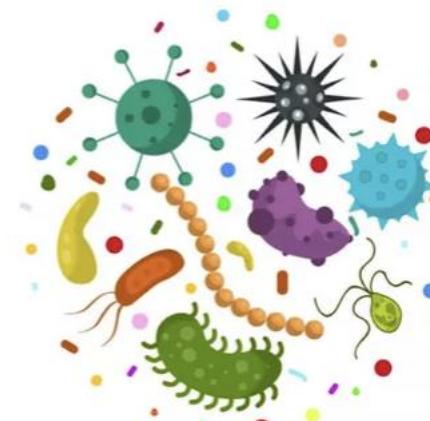
Course of action:



Targeted fever panel

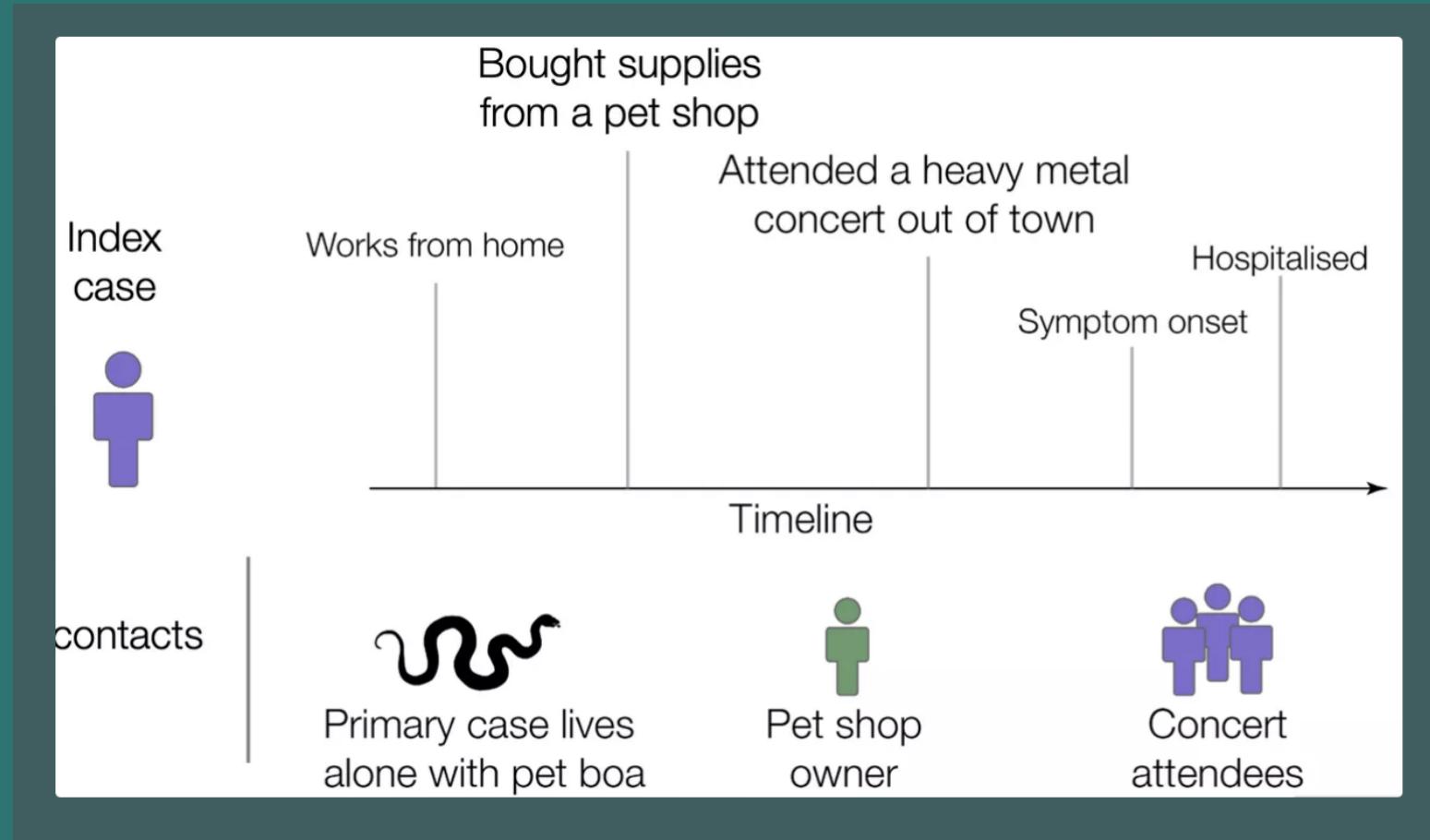


Epidemiological investigation/ questions



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
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Result: all negative

Results summary

Detected	Not-detected
None	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

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.

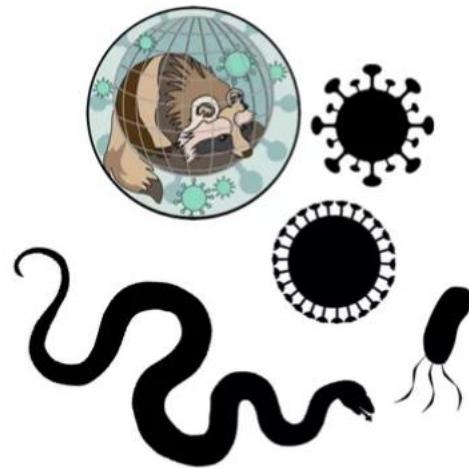
Executive Summary

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

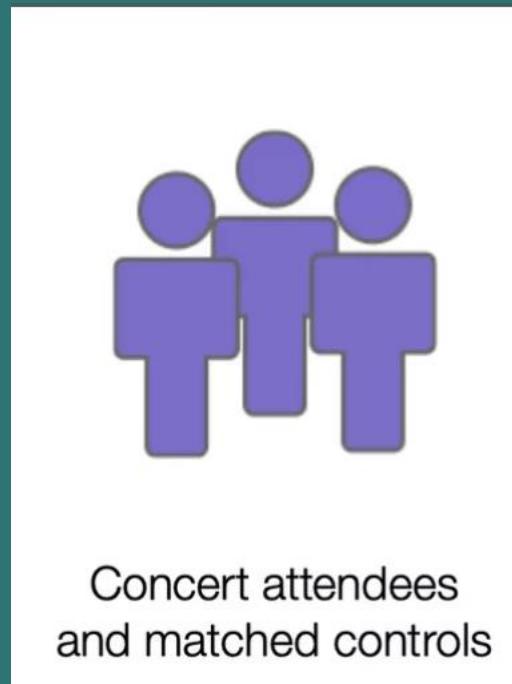


Environmental
sampling in pet shop



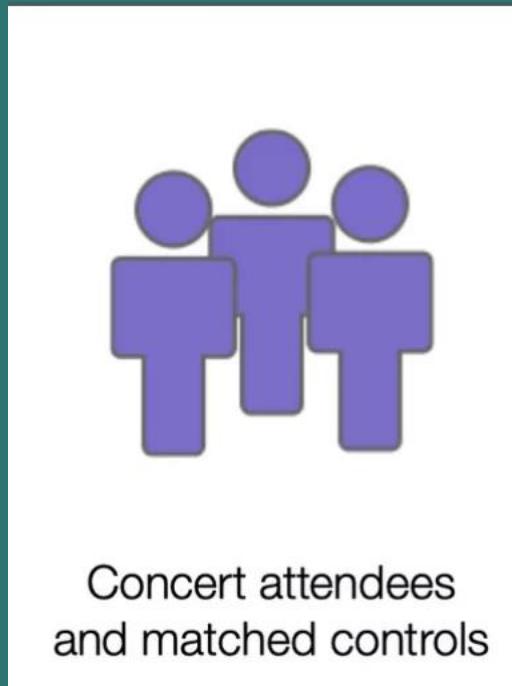
Concert attendees
and matched controls

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_3_title.html
https://artic-network.github.io/artic-live/gc/case_4_title.html

Heavy Metal Associated Controls

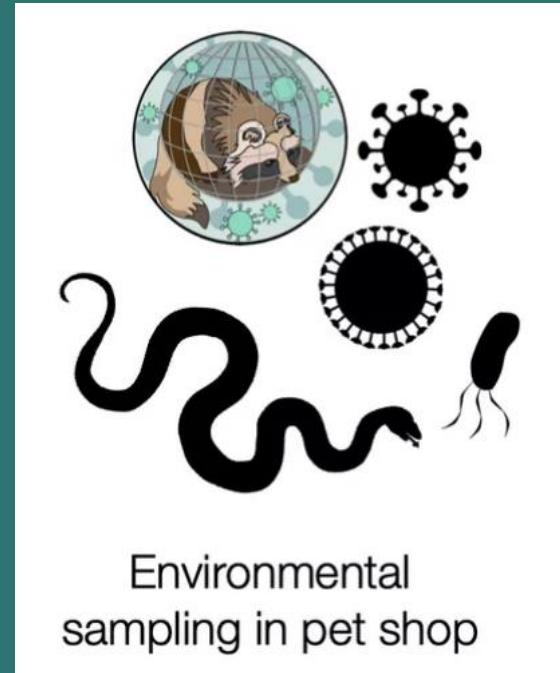


https://artic-network.github.io/artic-live/gc/control_1_title.html

https://artic-network.github.io/artic-live/gc/control_2_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

Wastewater Sampling



https://artic-network.github.io/artic-live/gc/wastewater_seq_title.html

What interventions are appropriate?

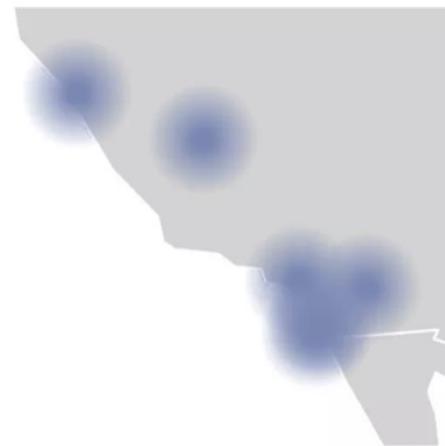
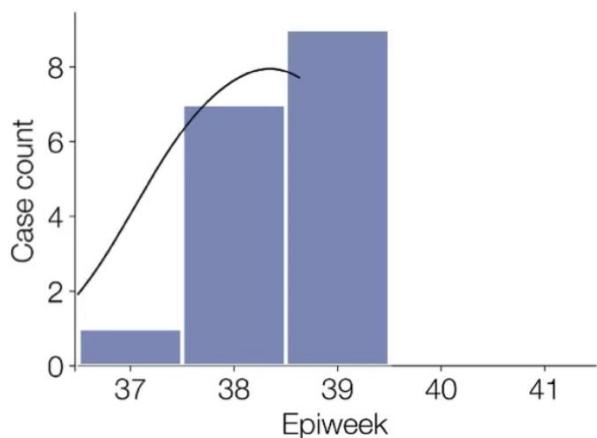
What pathogens are you ruling out and why?

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

Segment L - https://artic-network.github.io/artic-live/gc/segment_L.fasta.txt

Mammarenavirus draft genome

>segmentL

GCGCAGCGTGGAGCCTAGGCTTTACTTGACATT...

>segments

CGCGCGGTGGATCCTAGGGGTTTTACTTGAAC...

https://artic-network.github.io/artic-live/gc/segment_S.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 'Standard Nucleotide BLAST' search page. At the top, there's a navigation bar with links for 'blastn', 'blastp', 'blastx', 'tblastn', and 'tblastx'. Below the navigation is a search form with fields for 'Enter Query Sequence' (containing 'ATCG'), 'From' (containing '1'), 'To' (containing '2'), and 'Job Title'. There are also buttons for 'Choose Search Set', 'Database' (selected 'Standard databases (nr etc.)'), 'Organism' (selected 'Nucleotide collection (nt/nr)'), 'Program Selection' (selected 'Highly similar sequences (megablast)'), and 'Run BLAST'. A 'Help' link is visible at the bottom right of the search form.

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

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).



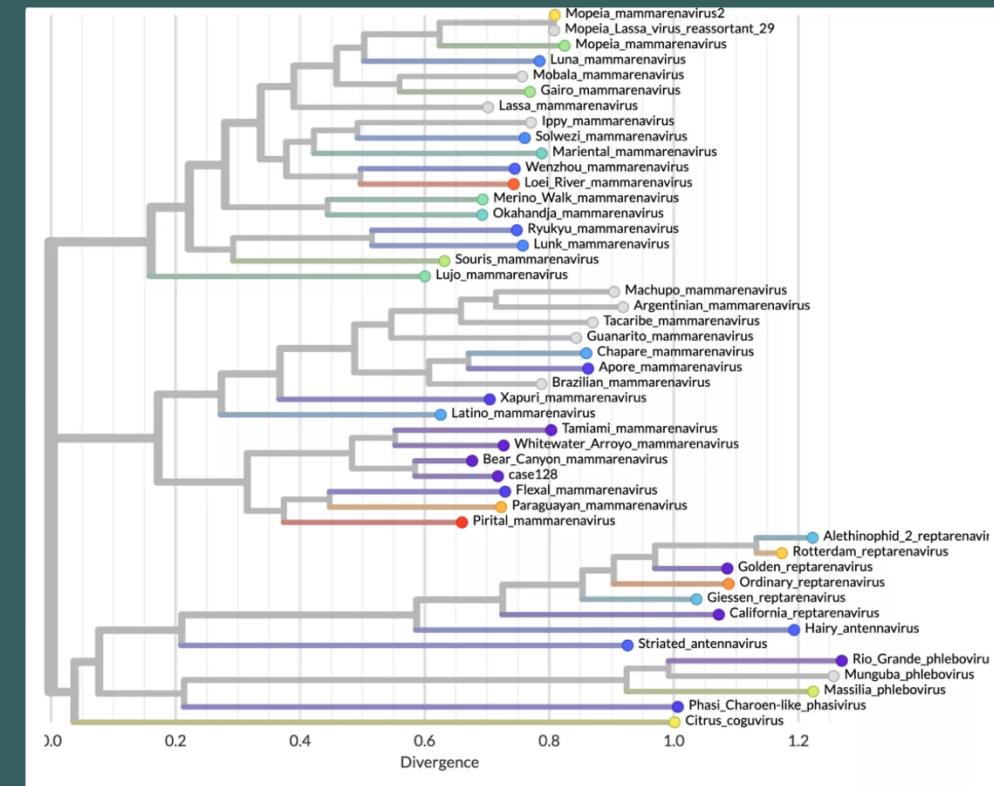
Evolutionary History Virus Phylogeny

Segment L:

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

Segment S:

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

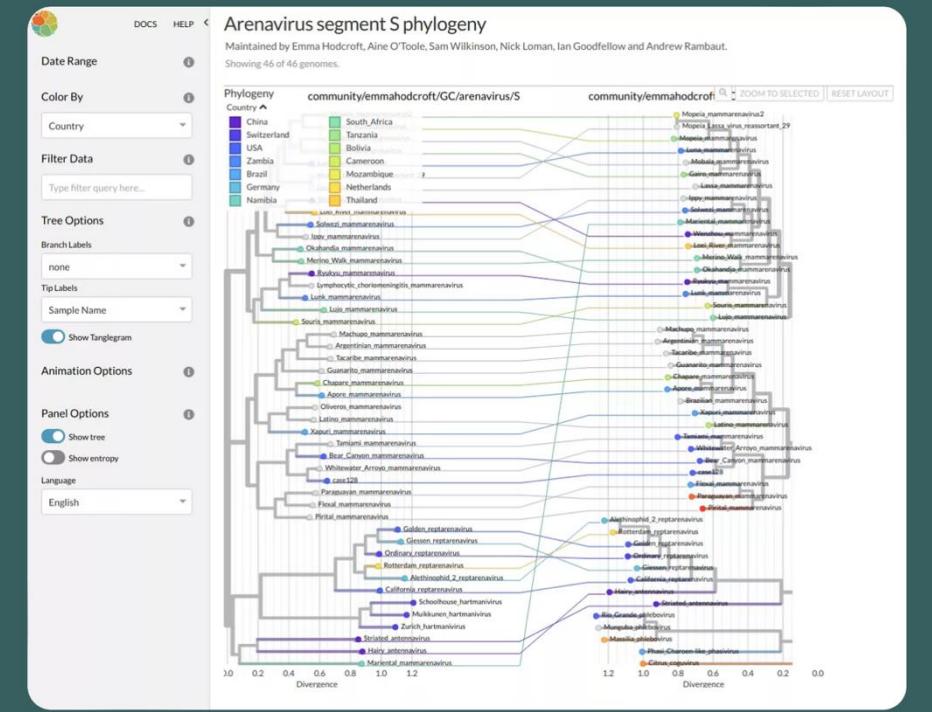


Segment L: <https://nextstrain.org/community/emmahodcroft/GC/arenavirus/L>

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>

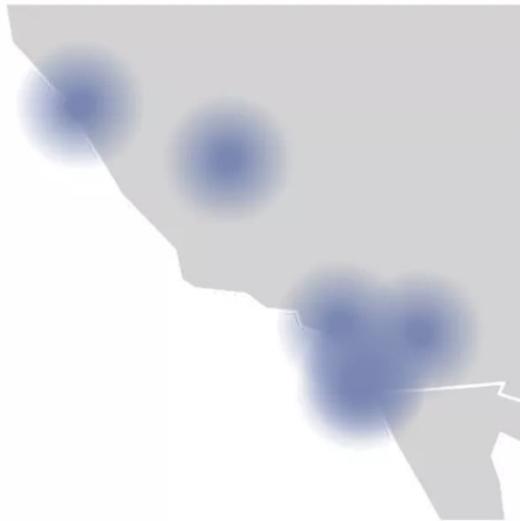
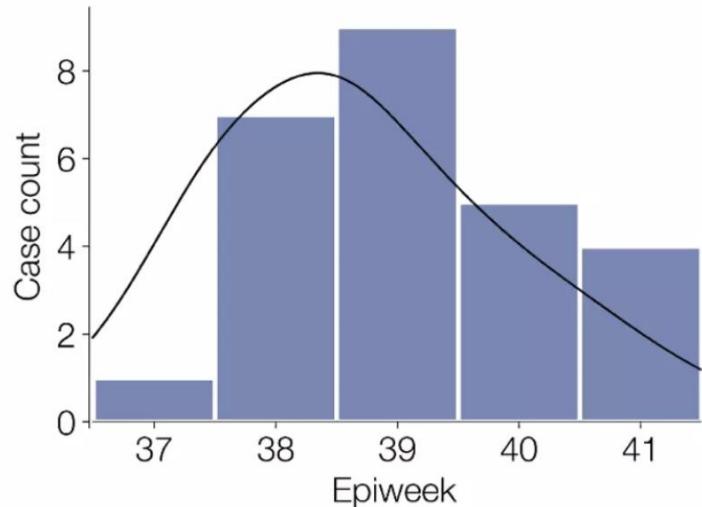
Advise policymakers on what this can tell us
about the novel virus

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".





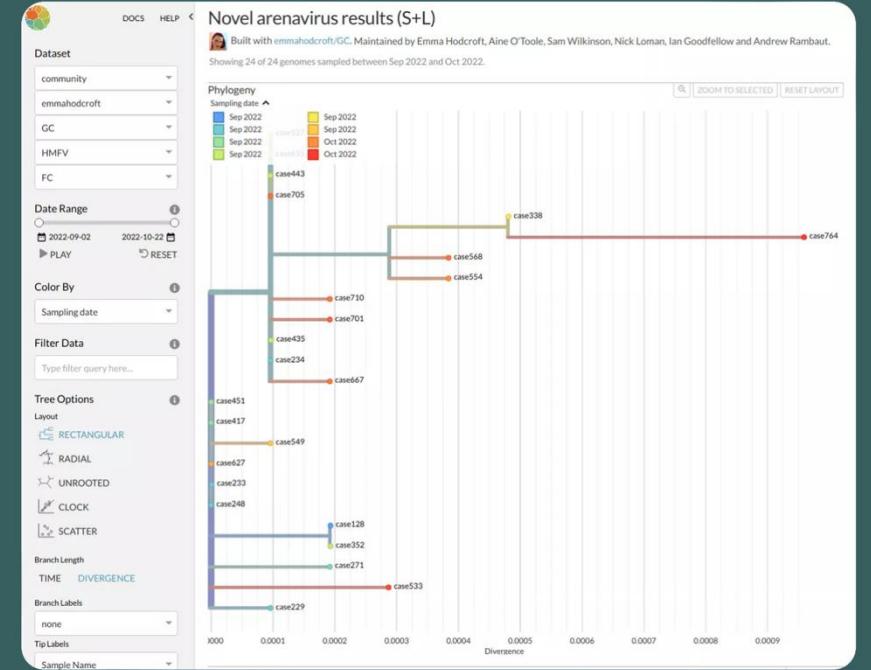
To: ProMED-mail

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)



auspice

nextstrain.org

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

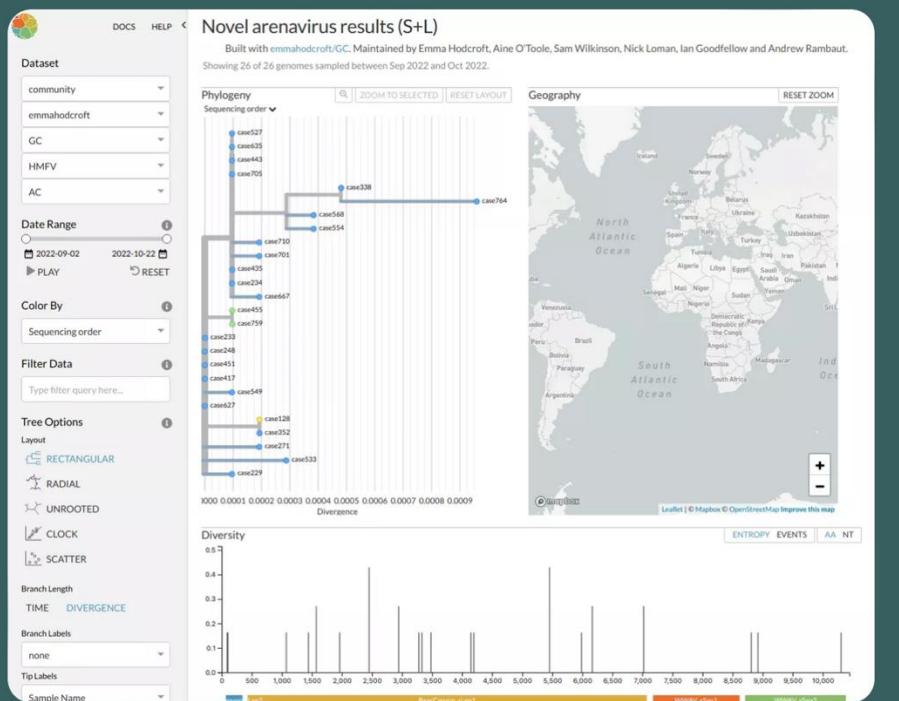
ACDC Alert : Two additional cases identified unlinked to a metal music event

Author: Donald McConnald PhD, ACDC outbreak investigation director.

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

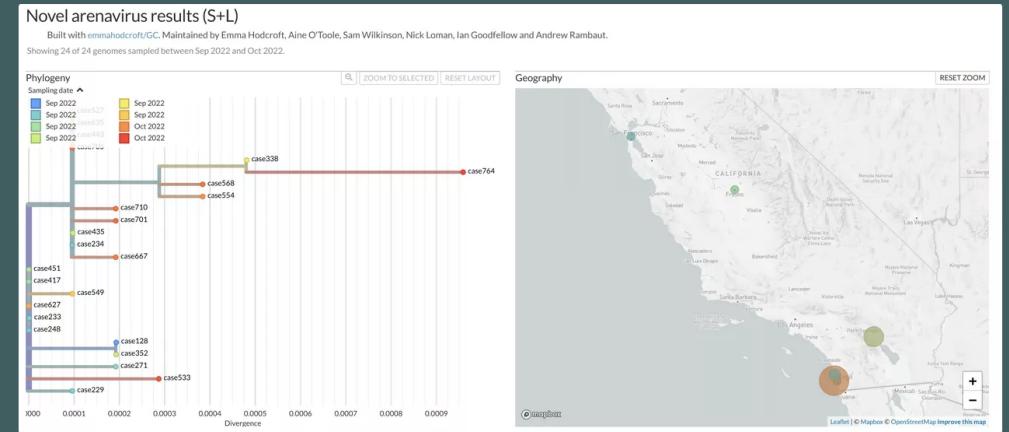


auspiceweb

nextstrain.org

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>

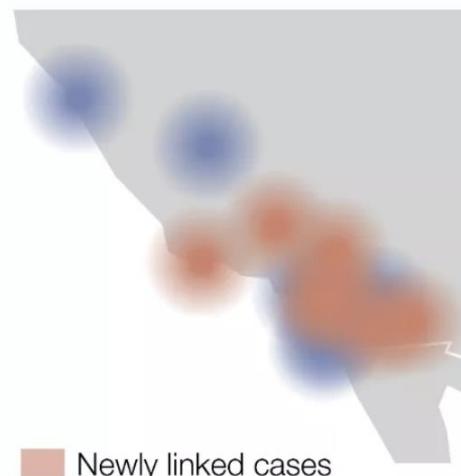
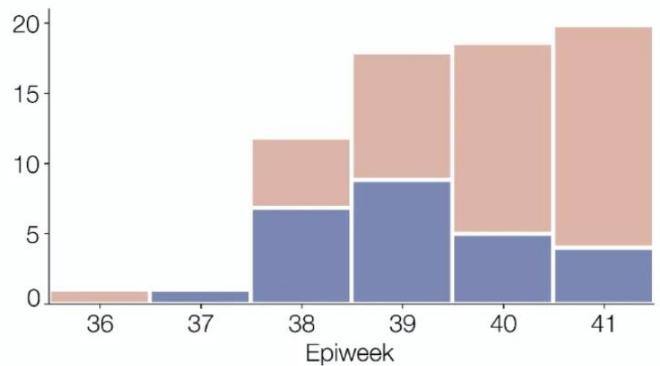
What can this tell us about the outbreak and
when it may have started?

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

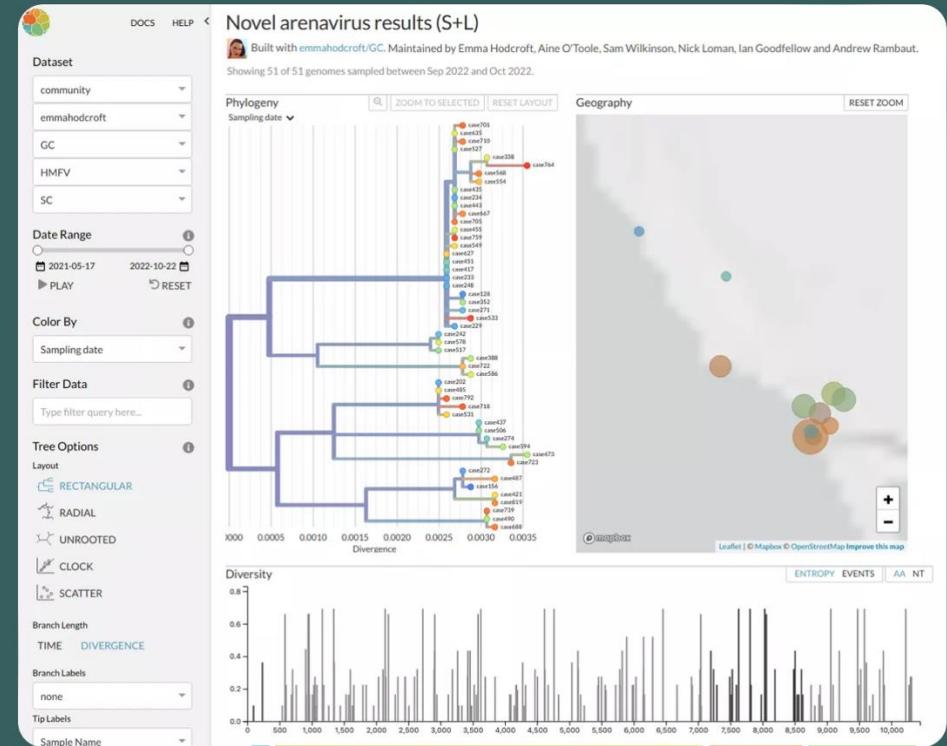
American Center for Disease Combatting

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/community/emmahodcroft/GC/HMFV/SC?p=grid>

BEAST phylodynamic report

Technical Report:

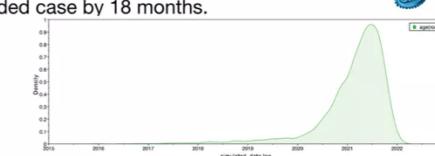
American Center for Disease Combatting



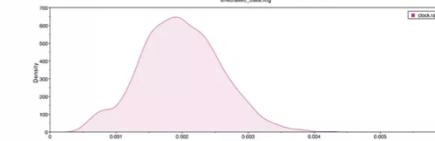
Executive Summary

Phylogenetic analysis has suggested the most recent common ancestor of known cases is early 2021, preceding the earliest recorded case by 18 months.

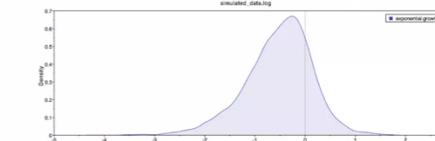
Age of tree root:
2021-02-14 [2019-09-17, 2022-01-11]



Evolutionary rate:
1.98E-3 [7.42E-4, 3.13E-3]



Exponential growth rate:
-0.5354 [-2.0074, 0.6598]



Does this change what we think about
the outbreak?

How do we stop this outbreak from
becoming a pandemic?

