



sajwilkinson

BIOM25 Metagenomics Practical

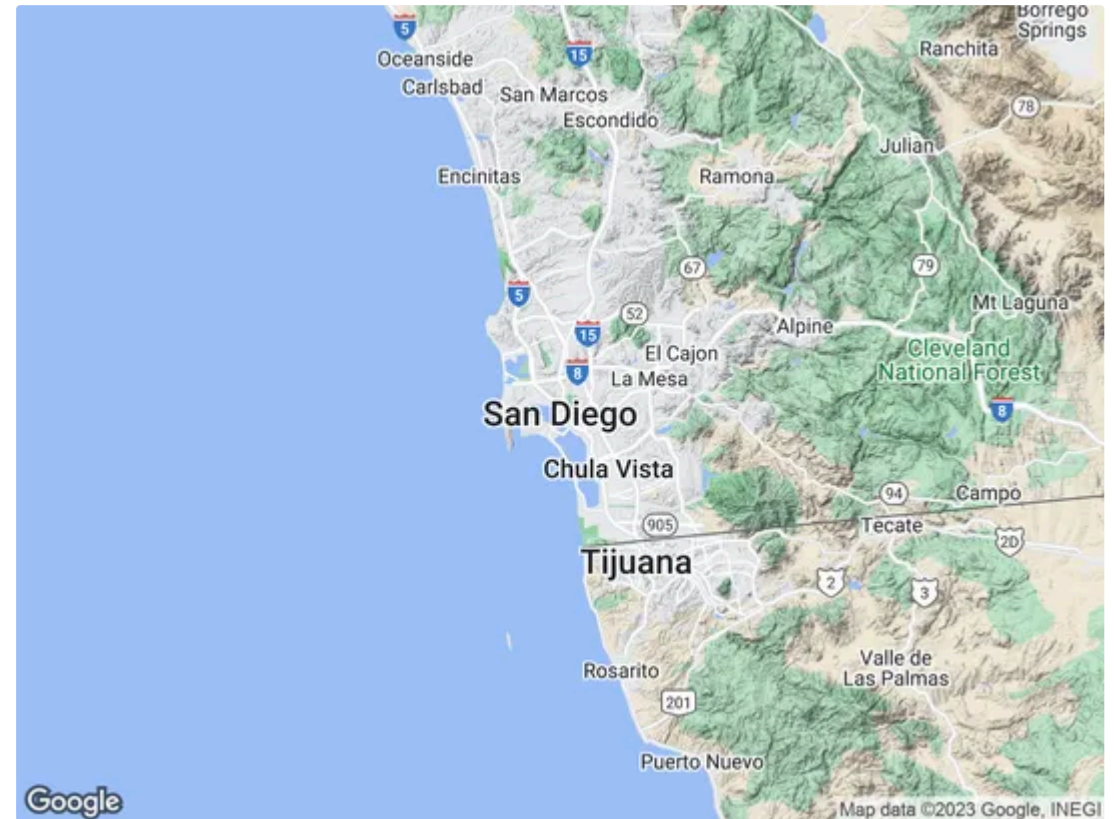
Good Morning!

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.

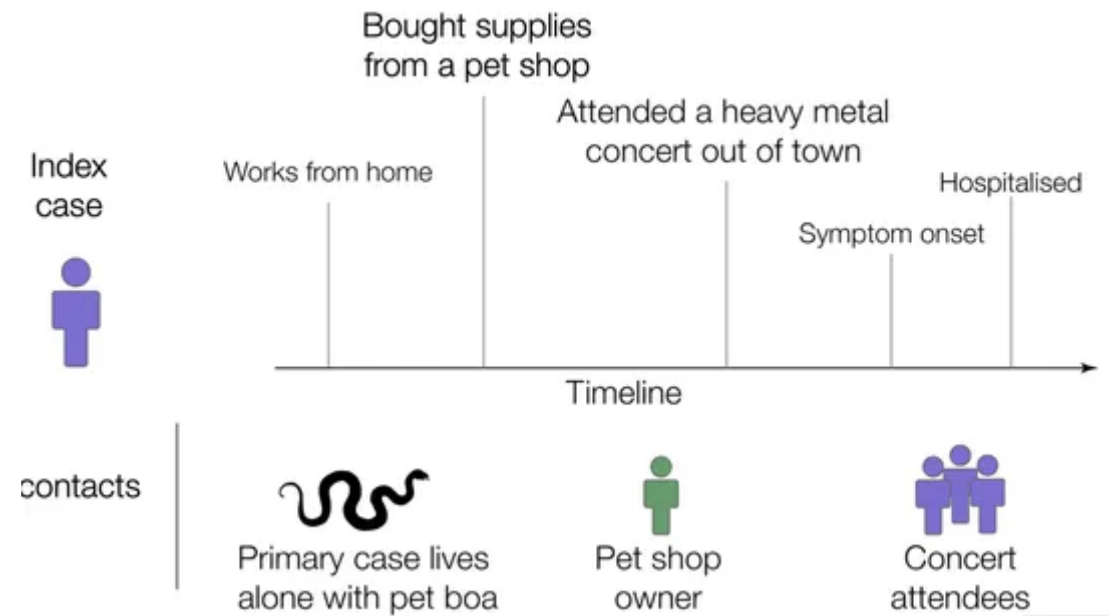


ACDC alert – undiagnosed febrile illness

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.



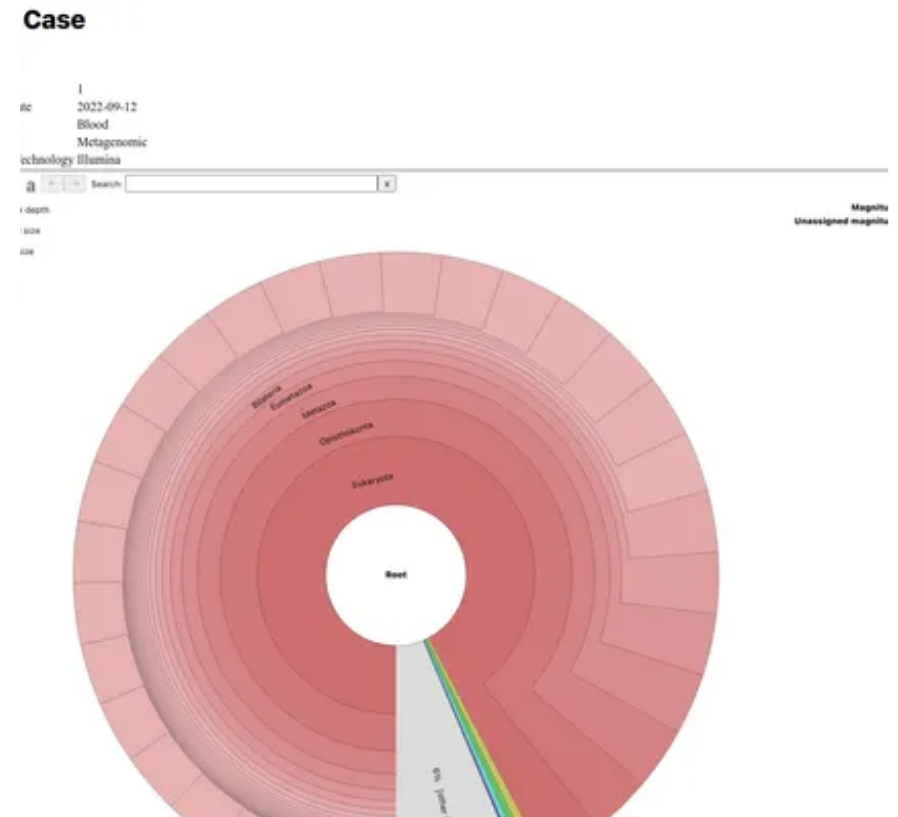
Enhanced epidemiology investigation



Global Fever Panel					
Sample ID:	CA-Vir-0001	Run date:	2022-09-21	Instrument:	VirDiag-5000-
Operator:	Bill	Run status:	complete	Protocol:	pro
		Controls:	passed		Whole blood
Result: all negative					
Results summary					
Detected None			Not-detected 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.



```
index case title
```

Raw Results

artic-network.github.io

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

Author: Donald McConnald PhD, ACDC outbreak investigation director.

Executive Summary

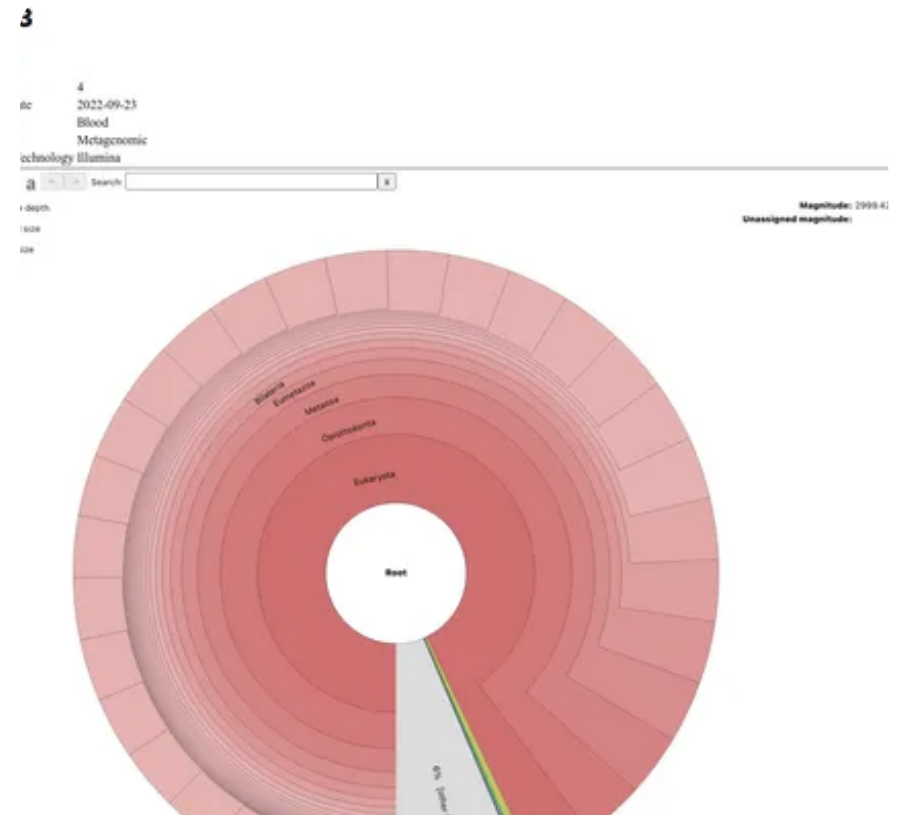
Officials at the ACDC, along with the local public health department, have identified suspected cases in the San Diego metropolitan area with similar clinical symptoms. Contact tracing efforts are currently underway and samples are being taken. At least 2 report attendance at the same concert as the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

Heavy metal-associated cases: Metalgenomics

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



Case 3

Raw Results

artic-network.github.io

Heavy metal-associated
controls:
Metalgenomics

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



Control 1

artic-network.github.io

Pet Shop Environmental Sequencing

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

t Shop Environmental Sequencing



Pet Shop Environmental Sequencing

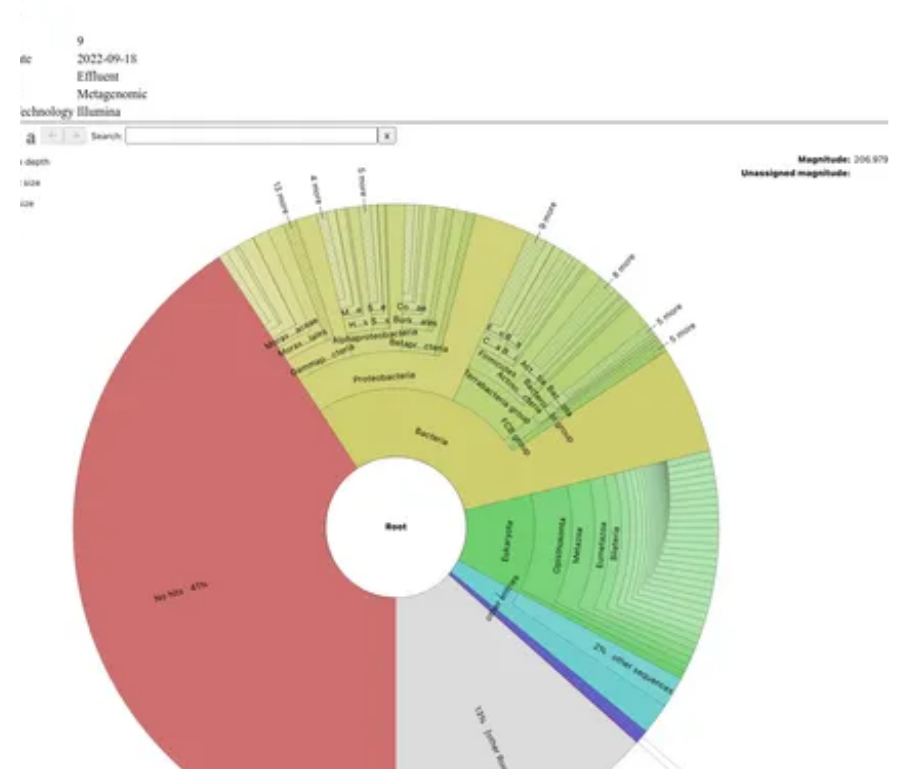
Raw Results

artic-network.github.io

Wastewater Sequencing

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

Wastewater Sequencing



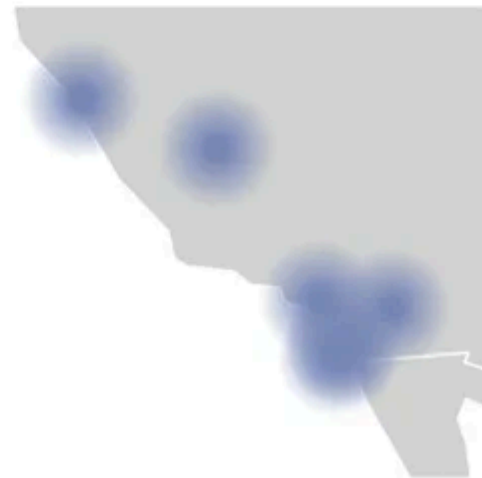
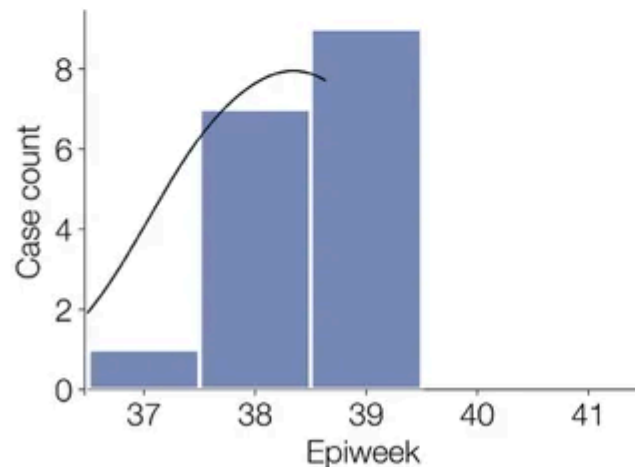
Wastewater Sequencing

artic-network.github.io

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.



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

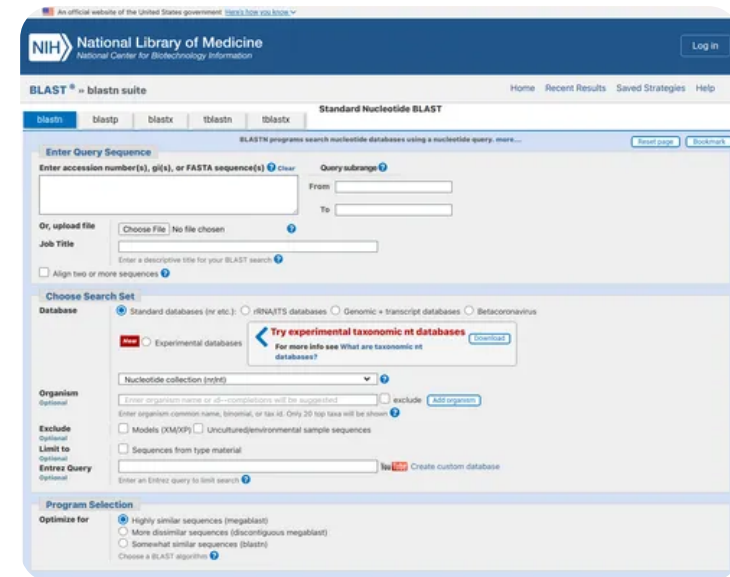
GCGCAGCGTGGAGCCTAGGCTTTACTTGACATT...

>segmentS

CGCGCGGTGGATCCTAGGGGTTTTTACTTGAAC...

NCBI Blast

Paste your sequence in the Query
Sequence box

The image shows the NCBI BLAST Standard Nucleotide BLAST web interface. At the top, there's a header for the National Library of Medicine. Below that, the 'BLAST' logo and 'blastn suite' are visible. The main section is titled 'Standard Nucleotide BLAST'. It includes a 'Query Sequence' input field with a 'Choose File' button and a 'No file chosen' status. There's also a 'Job Title' field. The 'Choose Search Set' section has radio buttons for 'Standard databases (nr etc.)', 'rRNA/ITS databases', 'Genomic + transcript databases', and 'Betacoronavirus'. A 'Try experimental taxonomic nt databases' link is present. The 'Organism' section has a dropdown menu and an 'Add organism' button. The 'Exclude' section has checkboxes for 'Models (XM/XP)', 'Uncultured/environmental sample sequences', and 'Sequences from type material'. The 'Limit to' section has a text input for 'Enter an Entrez query to limit search'. The 'Program Selection' section has radio buttons for 'Highly similar sequences (megablast)', 'More dissimilar sequences (discontiguous megablast)', and 'Somewhat similar sequences (blastn)'. A 'Choose a BLAST algorithm' link is at the bottom.

Nucleotide BLAST:
Search nucleotide
databases using a
nucleotide query

An official website of the United...

`blast.ncbi.nlm.nih.gov`

Segment L BLAST results

Organism	Blast Name	Score	Number of Hits	Description
root			110	
• Arenaviridae	viruses		109	
• • Mammarynavirus	viruses		106	
• • • Bear Canyon mammarynavirus	viruses	6715	4	Bear Canyon mammarynavirus hits
• • • Whitewater Arroyo mammarynavirus	viruses	2472	12	Whitewater Arroyo mammarynavirus hits
• • • Tamiami mammarynavirus	viruses	1027	6	Tamiami mammarynavirus hits
• • • Flexal mammarynavirus	viruses	541	1	Flexal mammarynavirus hits
• • • Pirital mammarynavirus	viruses	403	2	Pirital mammarynavirus hits
• • • Allpahuayo mammarynavirus	viruses	296	1	Allpahuayo mammarynavirus 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 mammarynavirus	viruses	143	1	Latino mammarynavirus hits
• • • Wufeng Niviventer niviventer mammarynavirus 1	viruses	104	1	Wufeng Niviventer niviventer mammarynavirus hits
• • • Wenzhou mammarynavirus	viruses	98.7	21	Wenzhou mammarynavirus hits
• • • Machupo mammarynavirus	viruses	98.7	9	Machupo mammarynavirus hits
• • • Lassa mammarynavirus	viruses	98.7	19	Lassa mammarynavirus hits
• • • Amapari virus	viruses	96.0	2	Amapari virus hits
• • • Xingyi virus	viruses	93.3	2	Xingyi virus hits
• • • Wenzhou Rattus losea mammarynavirus 1	viruses	92.4	1	Wenzhou Rattus losea mammarynavirus hits
• • • Rat mammarynavirus	viruses	91.5	2	Rat mammarynavirus hits
• • • Wenzhou Rattus tanezumii mammarynavirus 1	viruses	76.1	1	Wenzhou Rattus tanezumii mammarynavirus 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		120	
• Mammarenavirus	viruses		114	
• • Whitewater Arroyo mammarenavirus	viruses	3269	26	Whitewater Arroyo mammarenavirus hits
• • North American arenavirus	viruses	1691	18	North American arenavirus hits
• • Arenavirus AV 96010025	viruses	1427	1	Arenavirus AV 96010025 hits
• • Arenavirus H0380005	viruses	1210	1	Arenavirus H0380005 hits
• • Bear Canyon mammarenavirus	viruses	1095	18	Bear Canyon mammarenavirus hits
• • Tonto creek virus	viruses	1054	3	Tonto creek virus hits
• • Big brushy tank virus	viruses	1049	6	Big brushy tank virus hits
• • Catarina virus	viruses	1005	38	Catarina virus hits
• • Skinner Tank virus	viruses	899	1	Skinner Tank virus hits
• • Tamiami mammarenavirus	viruses	527	2	Tamiami mammarenavirus hits
• Arenavirus H0030026	viruses	967	1	Arenavirus H0030026 hits
• Black Mesa virus	viruses	891	4	Black Mesa virus hits
• Arenavirus H0030028	viruses	311	1	Arenavirus H0030028 hits

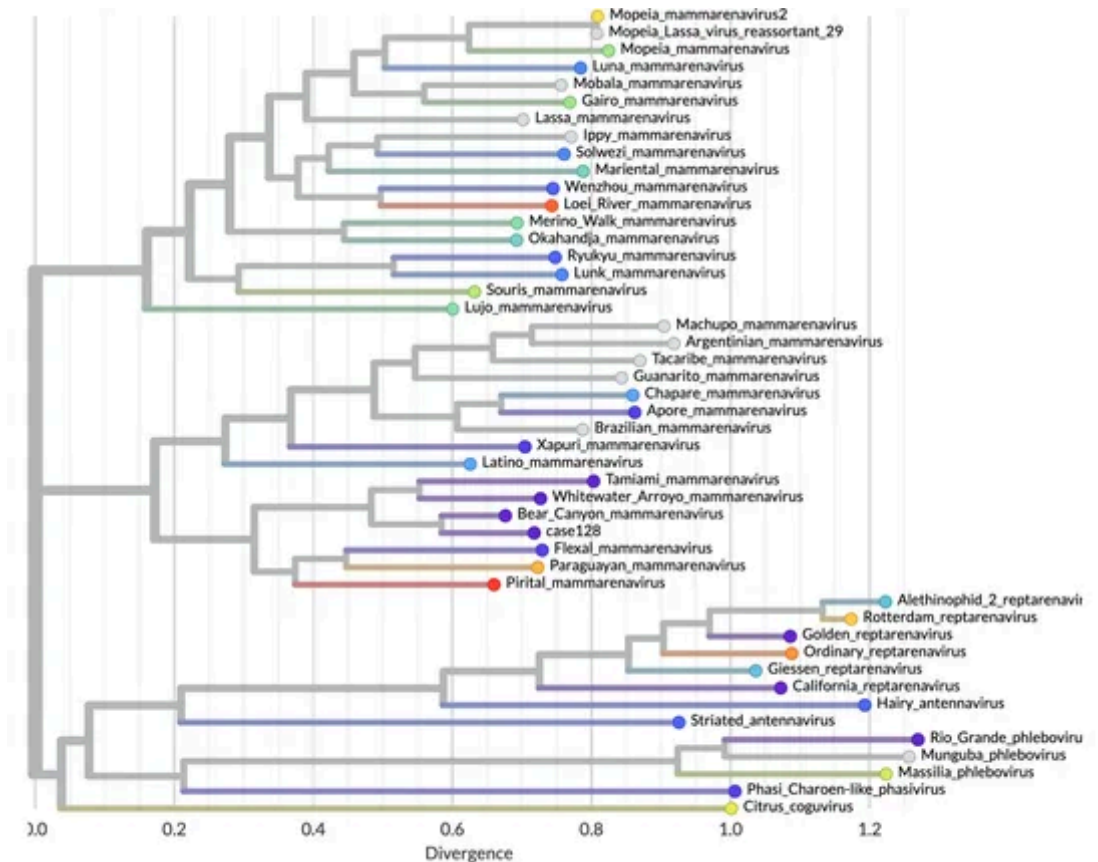
Evolutionary History Virus Phylogeny

Segment L:

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

Segment S:

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

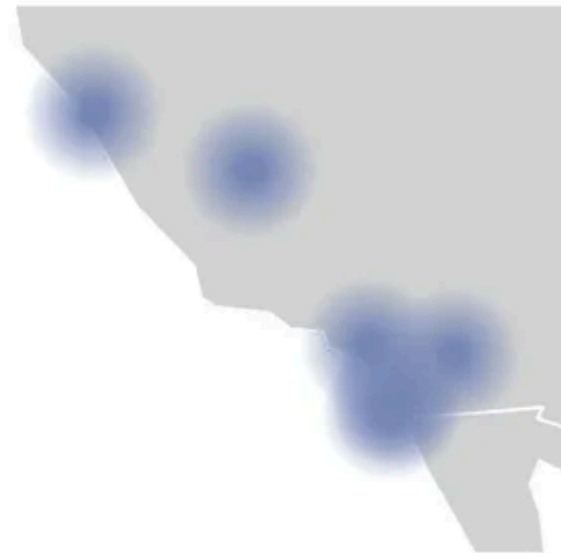
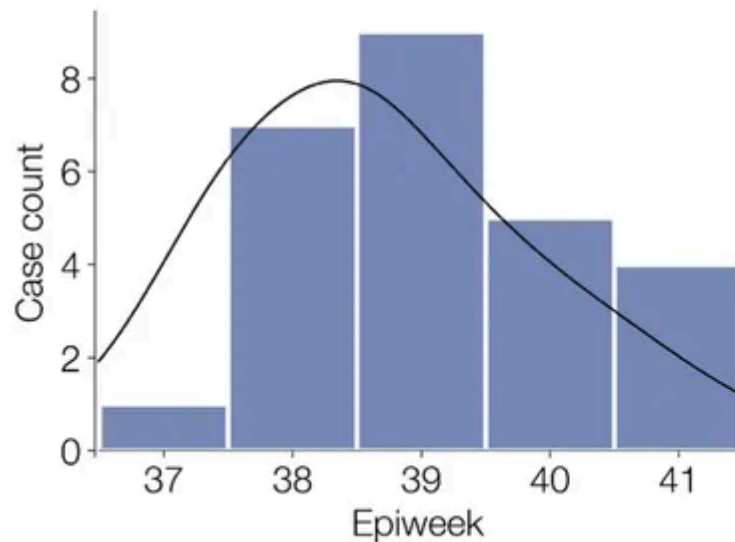


To: ProMED-mail

Subject: Further identification of heavy-metal related hemorrhagic fever

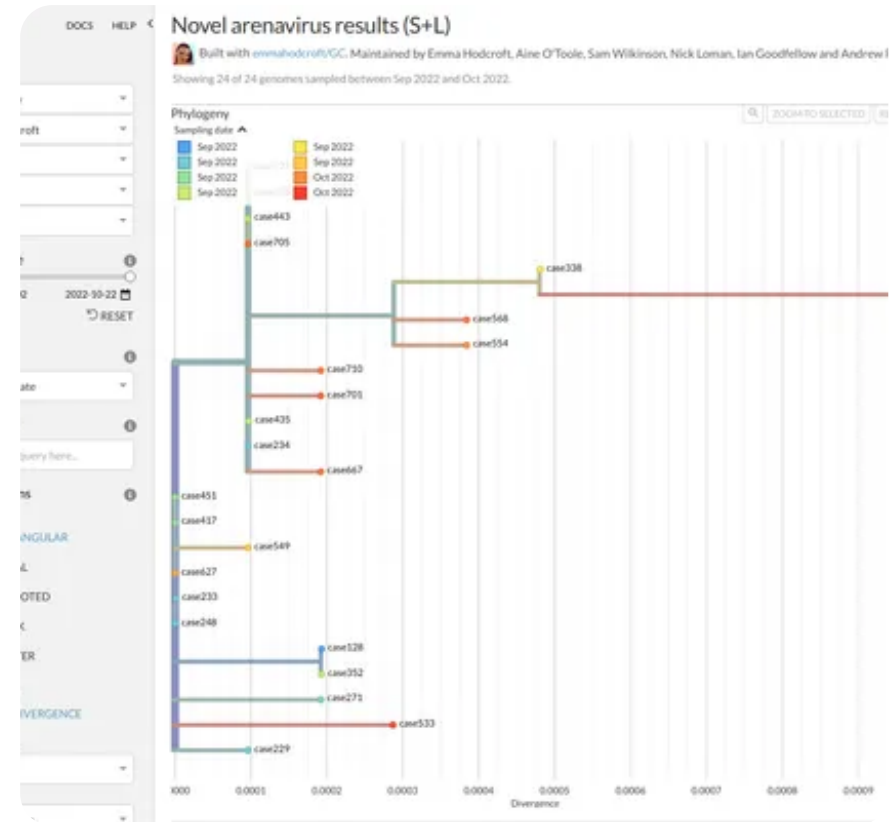
ACDC health officer Donald McConnell, PhD. has issued a statement regarding the HMFV outbreak. New cases have come to light that are secondary household cases, suggesting that human to human transmission is possible.

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".



Metal cluster sequencing

<https://nextstrain.org/community/emmahodcroft/GC/HMFV/FC>



auspice

nextstrain.org

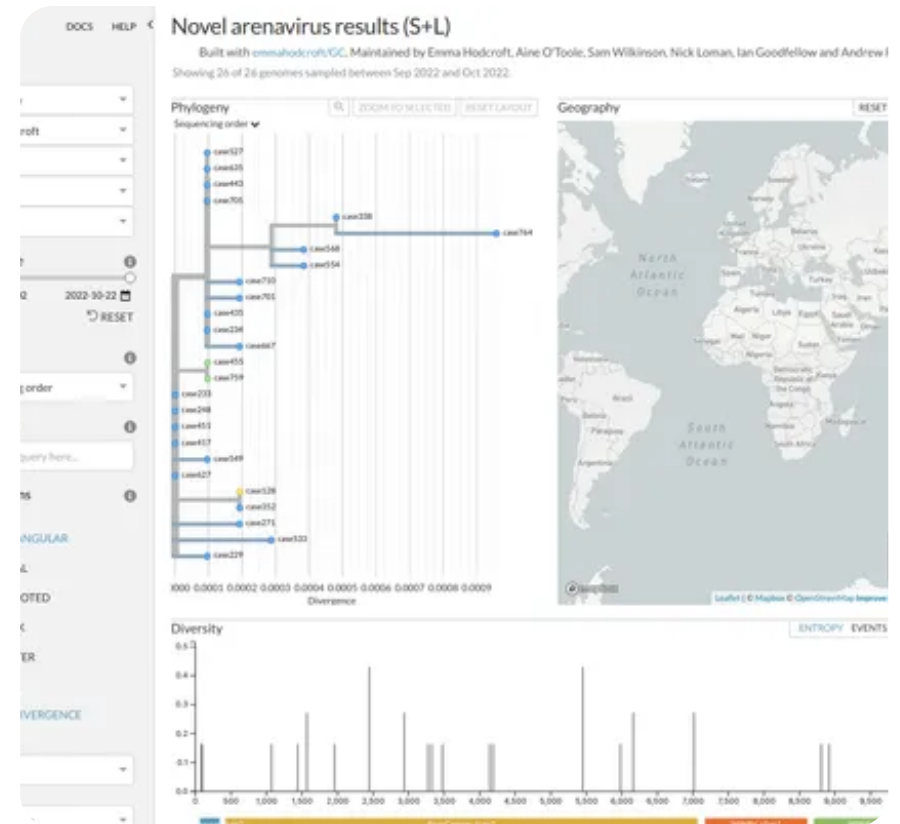
ACDC Alert : Two additional cases identified unlinked to a metal music event

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 Peakay, California.



Phylogenetic update 2



auspice

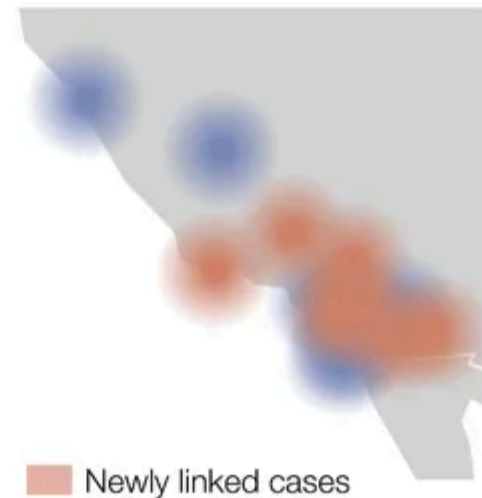
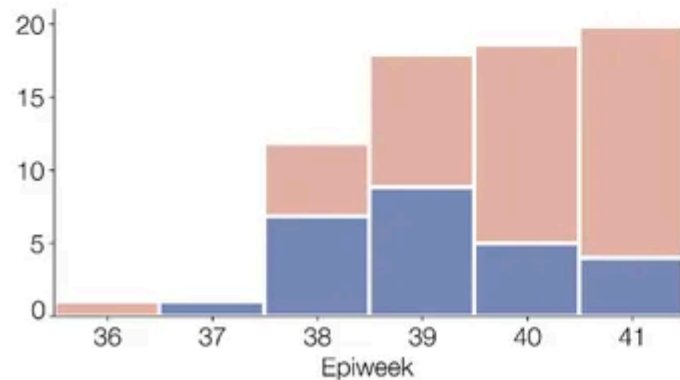
nextstrain.org

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

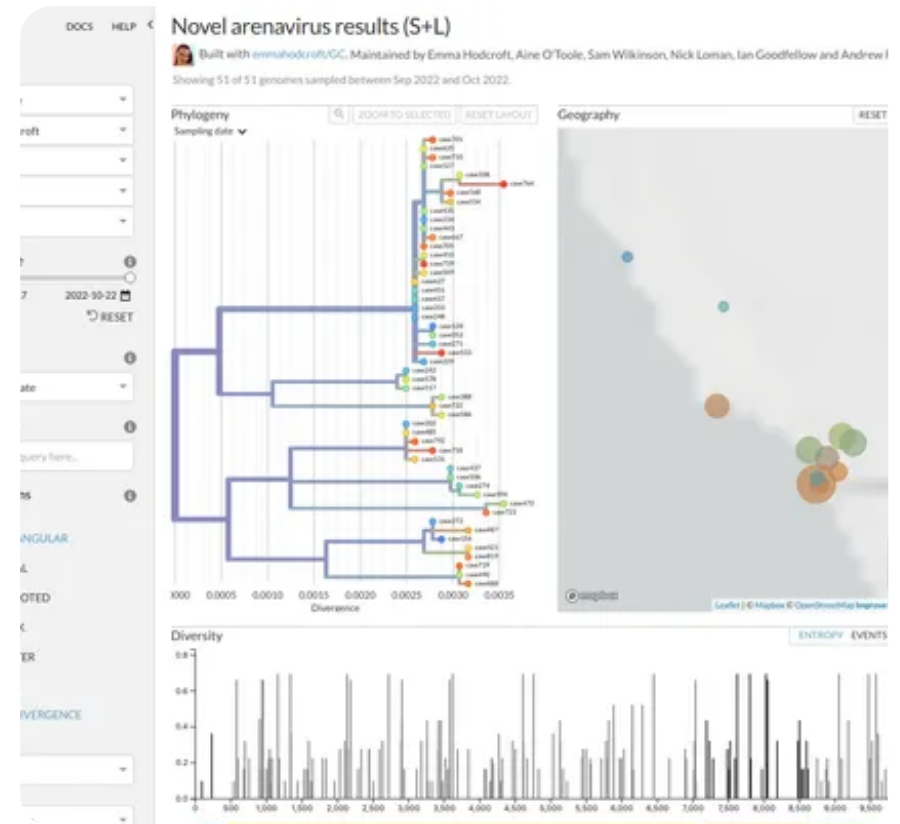
American Center for Disease Combatting

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



auspice

nextstrain.org