Sistema Socio Sanitario

Regione Lombardia

Fondazione IRCCS Policlinico San Matteo

ASST Pavia

ATS Pavia

UNIVERSITÀ DI PAVIA

GRAND ROUNDS CLINICI DEL MERCOLEDÌ

con il Policlinico San Matteo

Aula Magna "C. Golgi" & WEBINAR

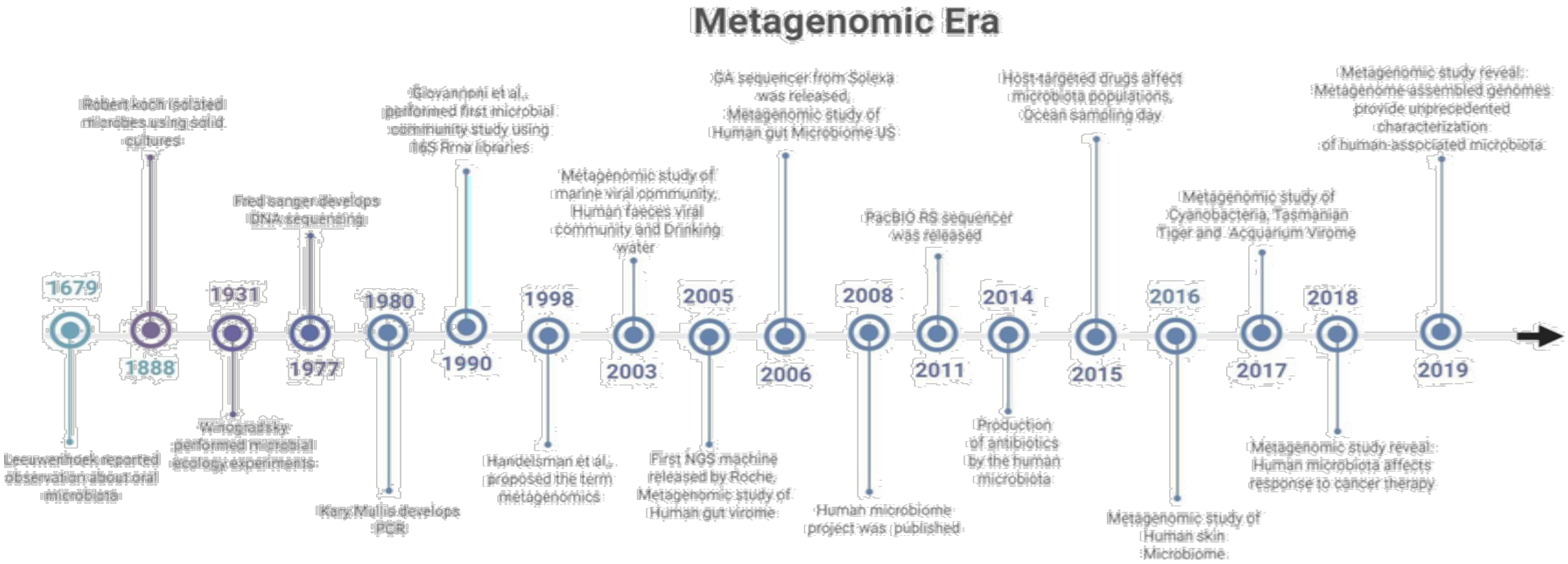
26/02/2025

Guglielmo Ferrari

S.C. Microbiologia e Virologia

L'approccio metagenomico: una nuova prospettiva nella microbiologia clinica

Navgire et al. Biological Procedures Online, 2022



1998. The term metagenome referenced the idea that a collection of genes sequenced from the environment could be analyzed in a way analogous to the study of a single genome.

2005. Kevin Chen and Lior Pachter defined metagenomics as "the application of modern genomics technique without the need for isolation and lab cultivation of individual species".

Definition

Metagenomics is the study of the structure and function of entire nucleotide sequences isolated and analyzed from all the organisms (typically microbes) in a bulk sample. Metagenomics is often used to study a specific community of microorganisms, such as those residing on human skin, in the soil or in a water sample.

Discovering the Role of Microbes in Disease

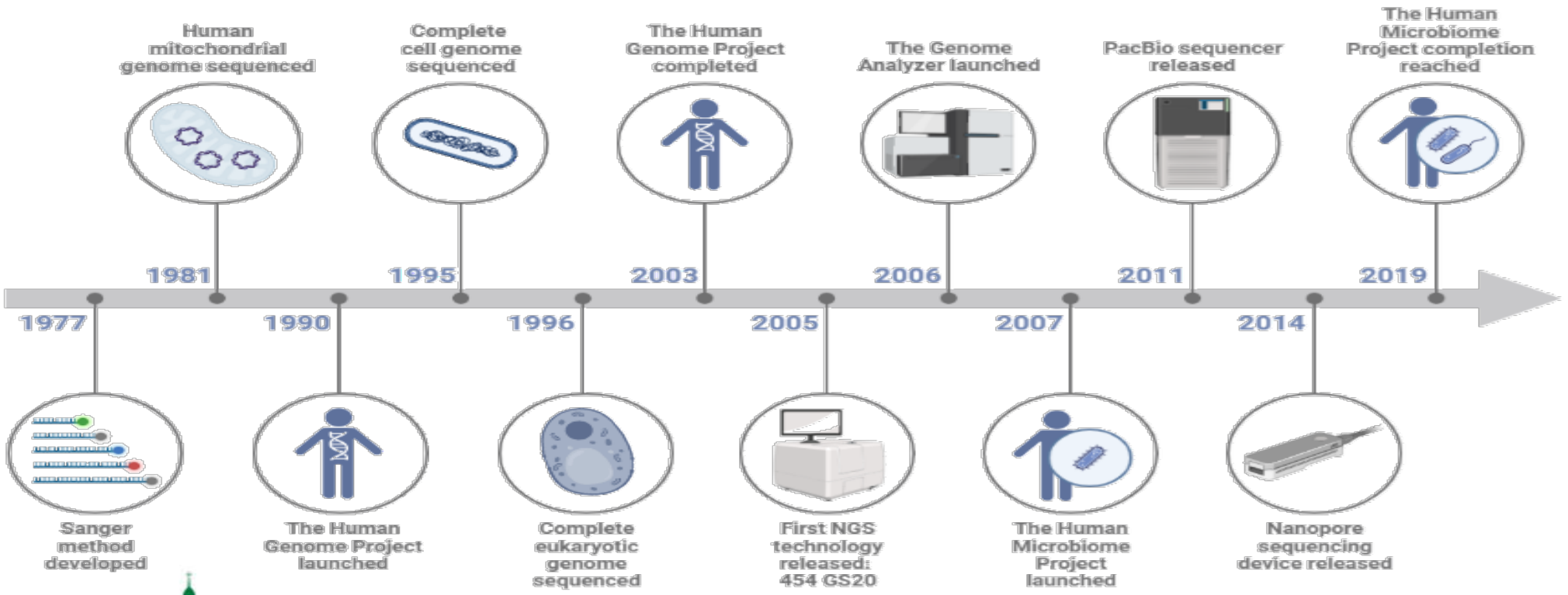
By analyzing the genes present in a metagenomic dataset, researchers can infer the metabolic pathways and potential functions of the microbial community. This helps us understand how microbes contribute to health and disease. Thus being able to study possible pathogen-host interactions and the potential role of environmental factors in pathogen transmission and persistence

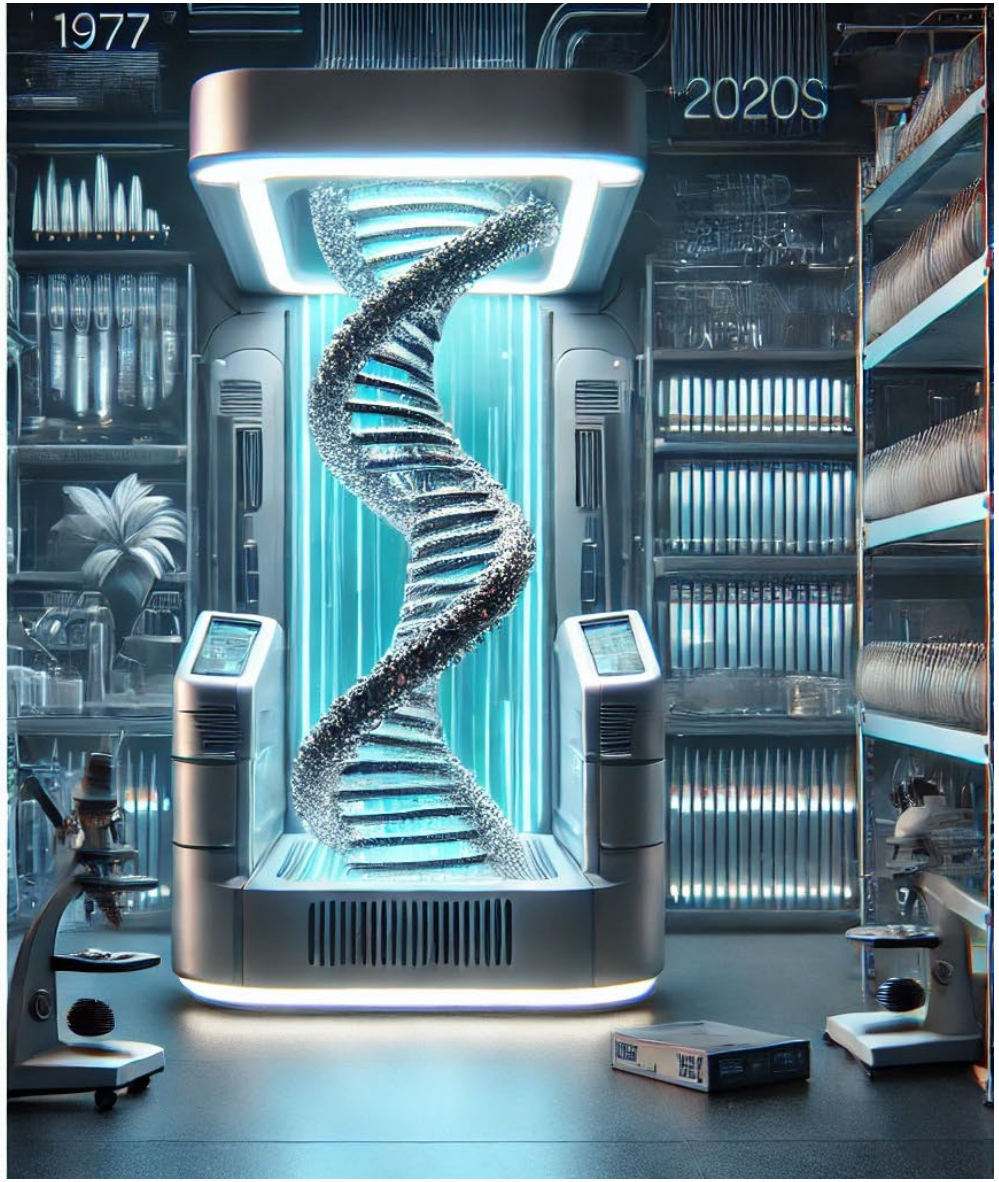
- Early Detection and Identification of Pathogens
  - known and novel pathogens
  - Overcoming traditional diagnostic methods that are unavailable
- Disease Diagnosis and Surveillance
  - tracking emergence and pathogen spread
  - informing public health interventions and response strategies
- Viral Evolution and Genetic Variation
  - virulence
  - Drug resistance
- Vaccine and Antiviral/Antibiotic Development

Uncovering Potential Therapeutic Targets

Personalized Medicine and Precision Outcome Interventions

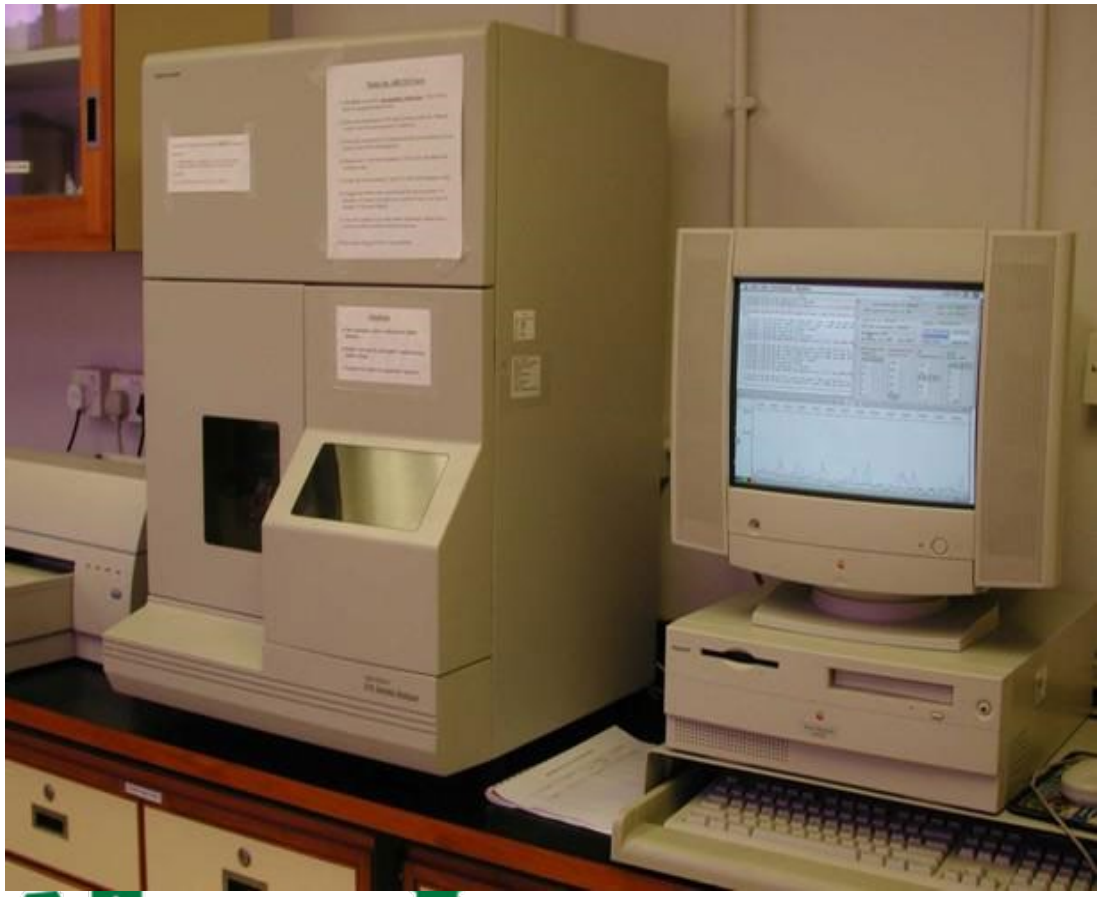
Sequencing technology timeline





GRAND ROUNDS CLINICI DEL MERCOLEDÌ

1990’S



2010’S

Different NGS approaches mean different results

NEW TECHNOLOGIES: METHODS AND APPLICATIONS

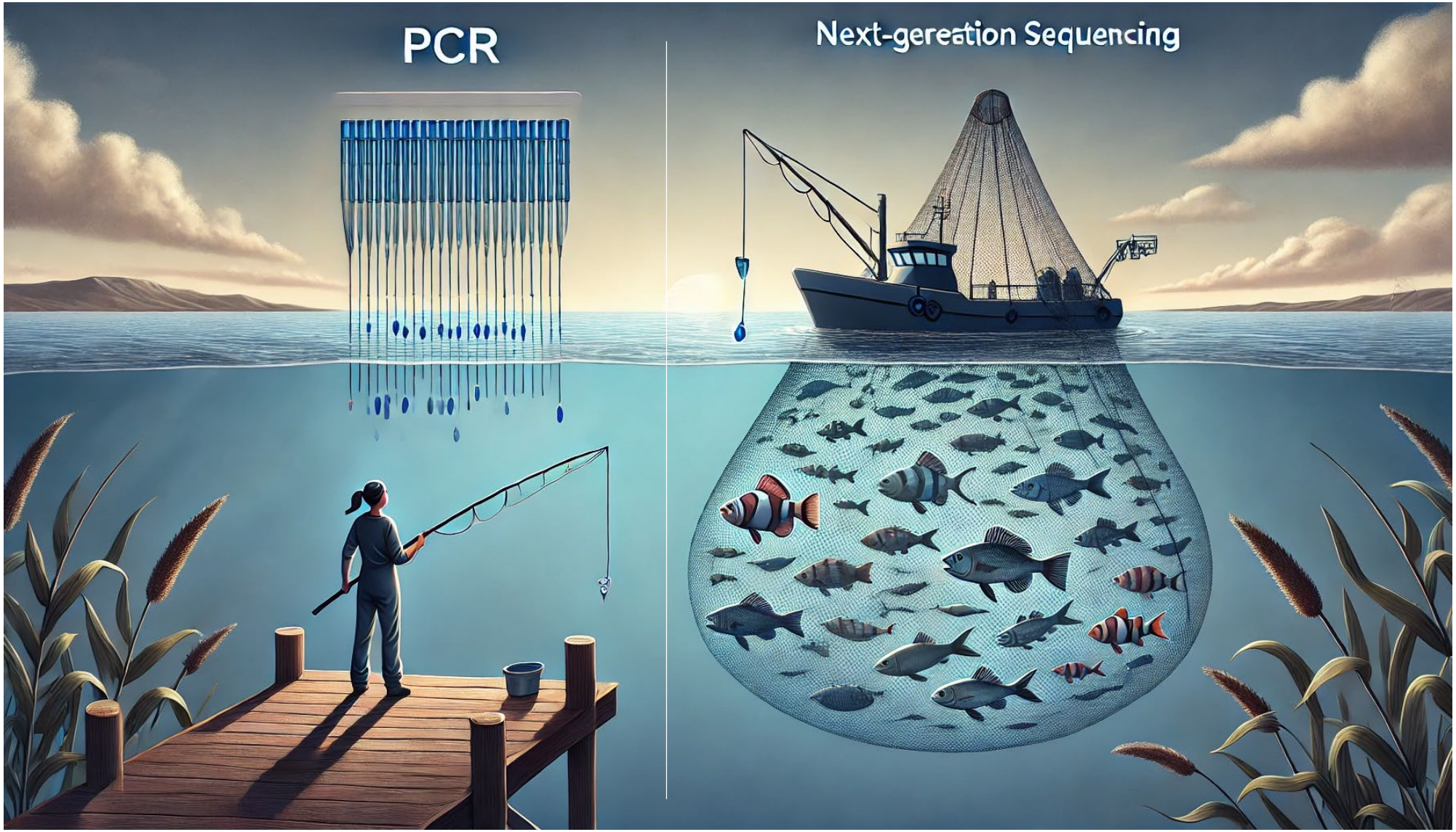
OPINION

Clinical and biological insights from viral genome sequencing

Charlotte J. Houldcroft, Mathew A. Beale and Judith Breuer

Method	Advantages	Disadvantages
Metagenomic sequencing	• Simple, cost-effective sample preparation • Can sequence novel or poorly characterized genomes • Effective in 'fishing' approaches to identify a potential underlying pathogen • Lower required number of PCR cycles causes few amplification mutations • Preservation of minor variant frequencies reflects *in vivo* variation • No primer or probe design required, which enables a rapid response to novel pathogens or sequence variants	• High sequencing cost to obtain sufficient data • Relatively low sensitivity to target pathogen • Coverage is proportional to viral load • High proportion of non-pathogen reads increases computational challenges • Incidental sequencing of human and off-target pathogens raises ethical and diagnostic issues
PCR amplification sequencing	• Tried and trusted well-established methods and trained staff • Highly specific; most sequencing reads will be pathogen-specific, which decreases sequencing costs • Highly sensitive, with good coverage even at low pathogen load • Relatively straightforward design and application of new primers for novel sequences	• Labour-intensive and difficult to scale for large genomes • Iterating standard PCRs across large genomes requires high sample volume • PCR reactions are subject to primer mismatch, particularly in poorly characterized or highly diverse pathogens, or those with novel variants • Limited ability to sequence novel pathogens • High number of PCR cycles may introduce amplification mutations • Uneven amplification of different PCR amplicons may influence minor variant and haplotype reconstruction
Target enrichment sequencing	• Single tube sample preparation that is suited to high-throughput automation and the sequencing of large genomes • Higher specificity than metagenomics decreases sequencing costs • Overlapping probes increases tolerance for individual primer mismatches • Fewer PCR cycles (than PCR amplification) limits the introduction of amplification mutations • Preservation of minor variant frequencies reflects *in vivo* variation	• High cost and technical expertise for sample preparation • Unable to sequence novel pathogens and requires well-characterized reference genomes for probe design • Sensitivity is comparable to PCR, but coverage is proportional to pathogen load; low pathogen load yields low or incomplete coverage • Cost and time to generate new probe sets limit a rapid response to emerging and novel viruses

Metagenomics (mNGS) as diagnostic application



Stringent Reporting Criteria

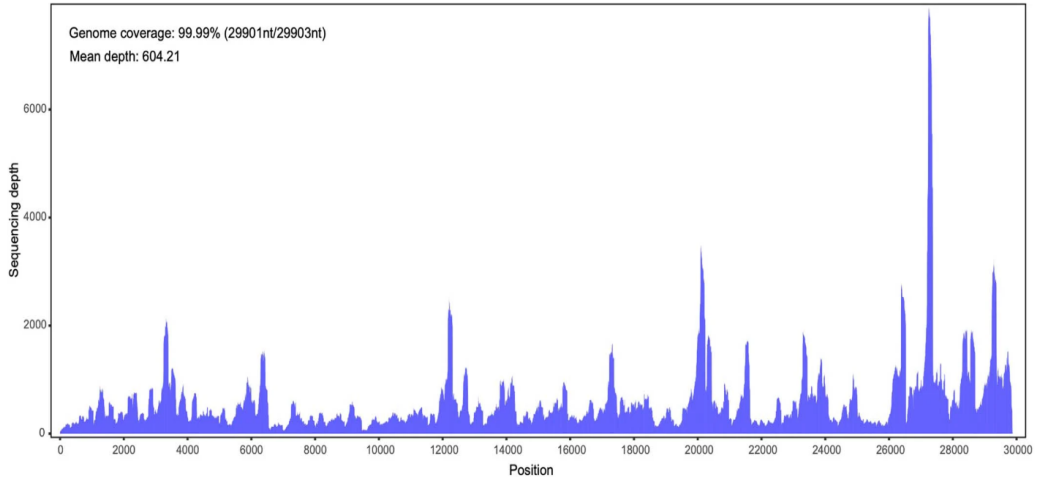
- Reads Per Million (RPM) value --> $RPM = \frac{RAT * 10^6}{TRR}$
- RPM-ratio (RPM-r) metric (>10 for bacteria) --> $RPM - r = \frac{RPM}{RPMc}$
- Absence form the negative control
- Confirmatory mapping with reads mapping to ≥ 3 non-overlapping regions

Article | Open access | Published: 03 February 2020

A new coronavirus associated with human respiratory disease in China

Fan Wu, Su Zhao, Bin Yu, Yan-Mei Chen, Wen Wang, Zhi-Gang Song, Yi Hu, Zhao-Wu Tao, Jun-Hua Tian, Yuan-Yuan Pei, Ming-Li Yuan, Yu-Ling Zhang, Fa-Hui Dai, Yi Liu, Qi-Min Wang, Jiao-Jiao Zheng, Lin Xu, Edward C. Holmes & Yong-Zhen Zhang

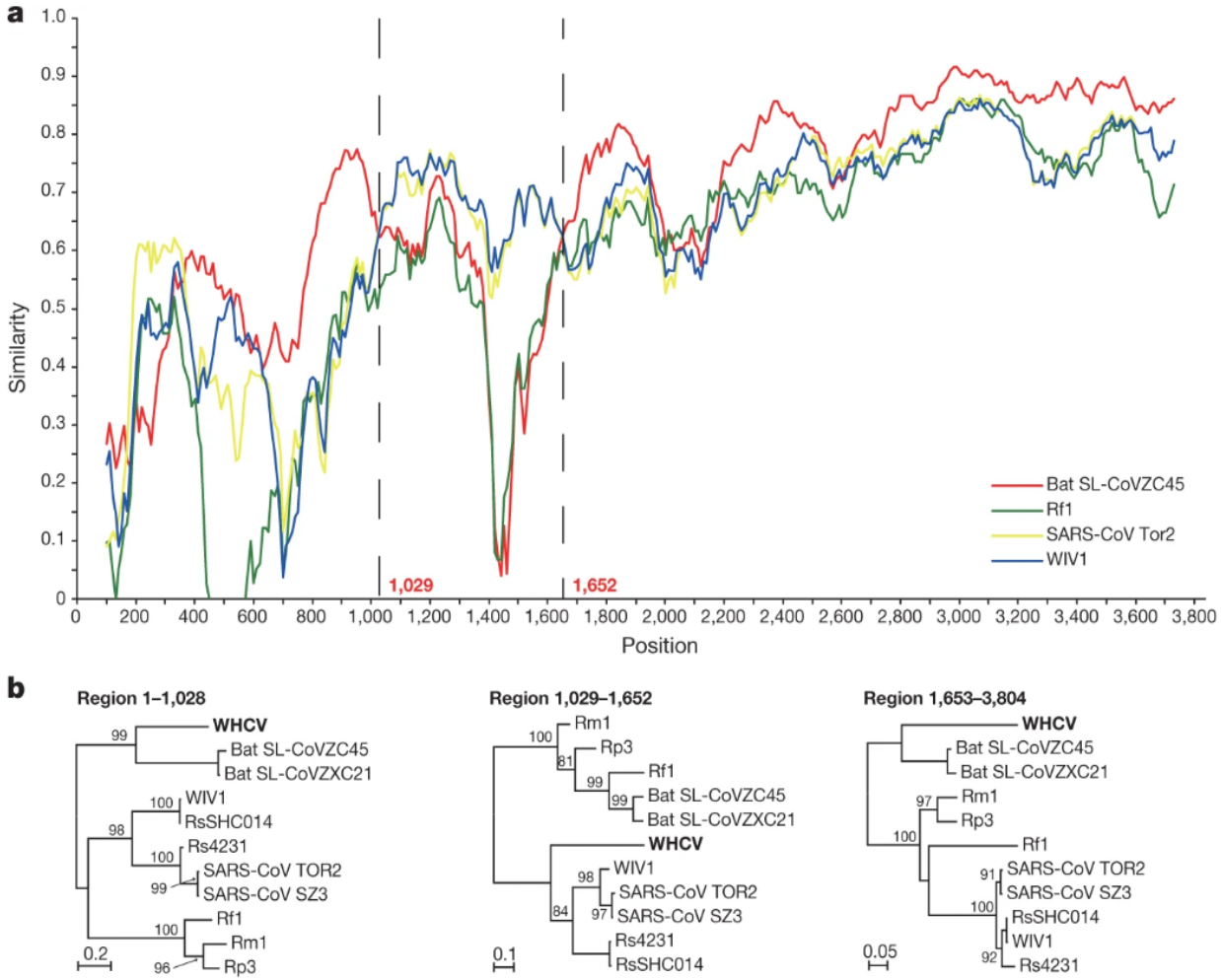
Nature **579**, 265–269 (2020) | Cite this article



The histograms show the coverage depth per base of the WHCV genome. The mean sequencing depth of the WHCV genome was 604.21 nt.

Fig. 3: Possible recombination events in the *S* gene of sarbecoviruses.

From: A new coronavirus associated with human respiratory disease in China

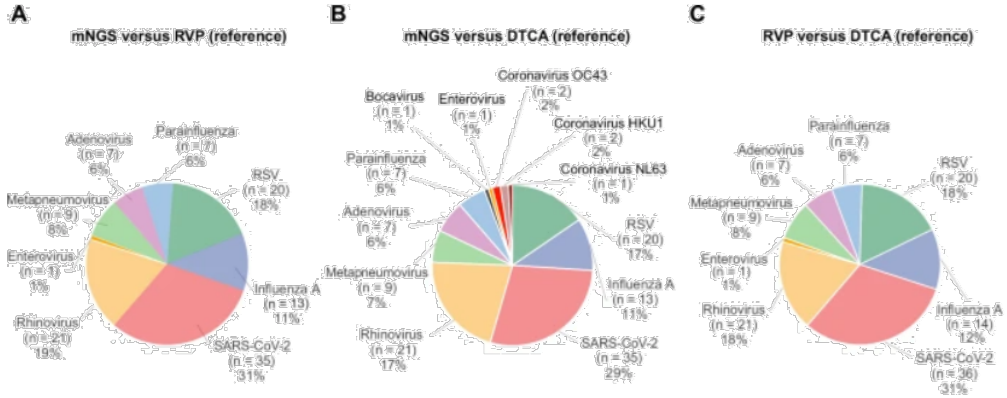


Nat Commun. 2024 Nov 12;15(1):9016. doi: 10.1038/s41467-024-51470-y.

Laboratory validation of a clinical metagenomic next-generation sequencing assay for respiratory virus detection and discovery

Jessica Karielle Tan [# 1 2], Venice Servellita [# 1 2], Doug Stryke [# 1 2], Emily Kelly [1], Jessica Streithorst [1], Nanami Sumimoto [1 2], Abiodun Foresythe [1 2], Hee Jae Huh [1 2 3], Jenny Nguyen [1 2], Miriam Oseguera [1 2], Noah Brazer [1 2], Jack Tang [1 2], Danielle Ingebrigtsen [1], Becky Fung [1], Helen Reyes [1], Melissa Hillberg [1], Alice Chen [4], Hugo Guevara [4], Shigeo Yagi [4], Christina Morales [4], Debra A Wadford [4], Peter M Mourani [5], Charles R Langelier [6 7], Mikael de Lorenzi-Tognon [1 2], Patrick Benoit [1 2], Charles Y Chiu [8 9 10 11]

Fig. 6: Accuracy evaluation for the mNGS assay.



	RVP PCR Positive	RVP PCR Negative
mNGS Positive	103	5
mNGS Negative	7	76

Sensitivity: 93.6%
Specificity: 93.8%
Accuracy: 93.7%

	DTCA Positive	DTCA Negative
mNGS Positive	110.5	1.5
mNGS Negative	1.5	76.5

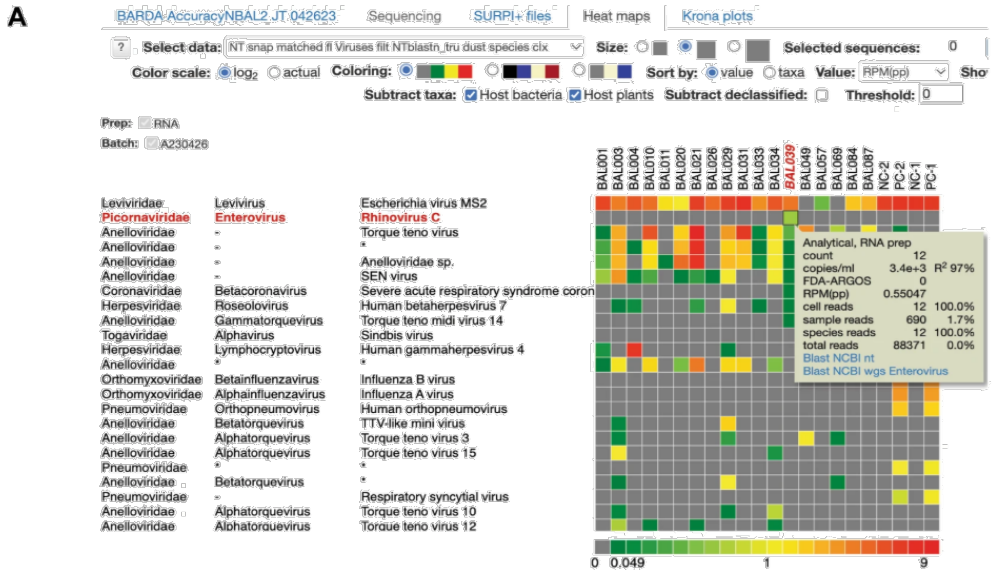
mNGS PPA: 98.7%
mNGS NPA: 98.1%
mNGS OPA: 97.9%

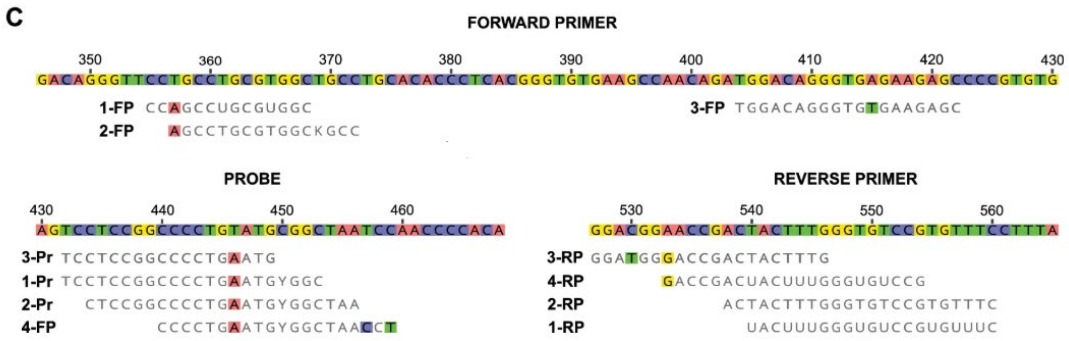
	DTCA Positive	DTCA Negative
RVP PCR Positive	104	5.5
RVP PCR Negative	4	77.5

RVP PPA: 96.3%
RVP NPA: 93.4%
RVP OPA: 95.0%

Fig. 7: In-depth analysis of a rhinovirus C detection by mNGS that was discrepant with RT-PCR.

From: Laboratory validation of a clinical metagenomic next-generation sequencing assay for respiratory virus detection and discovery



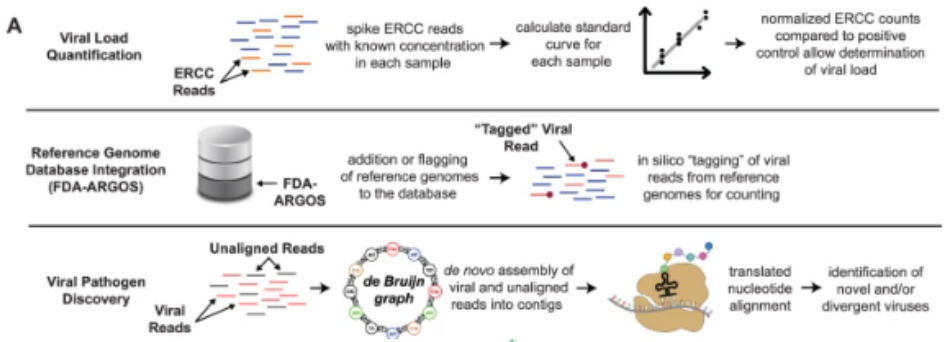


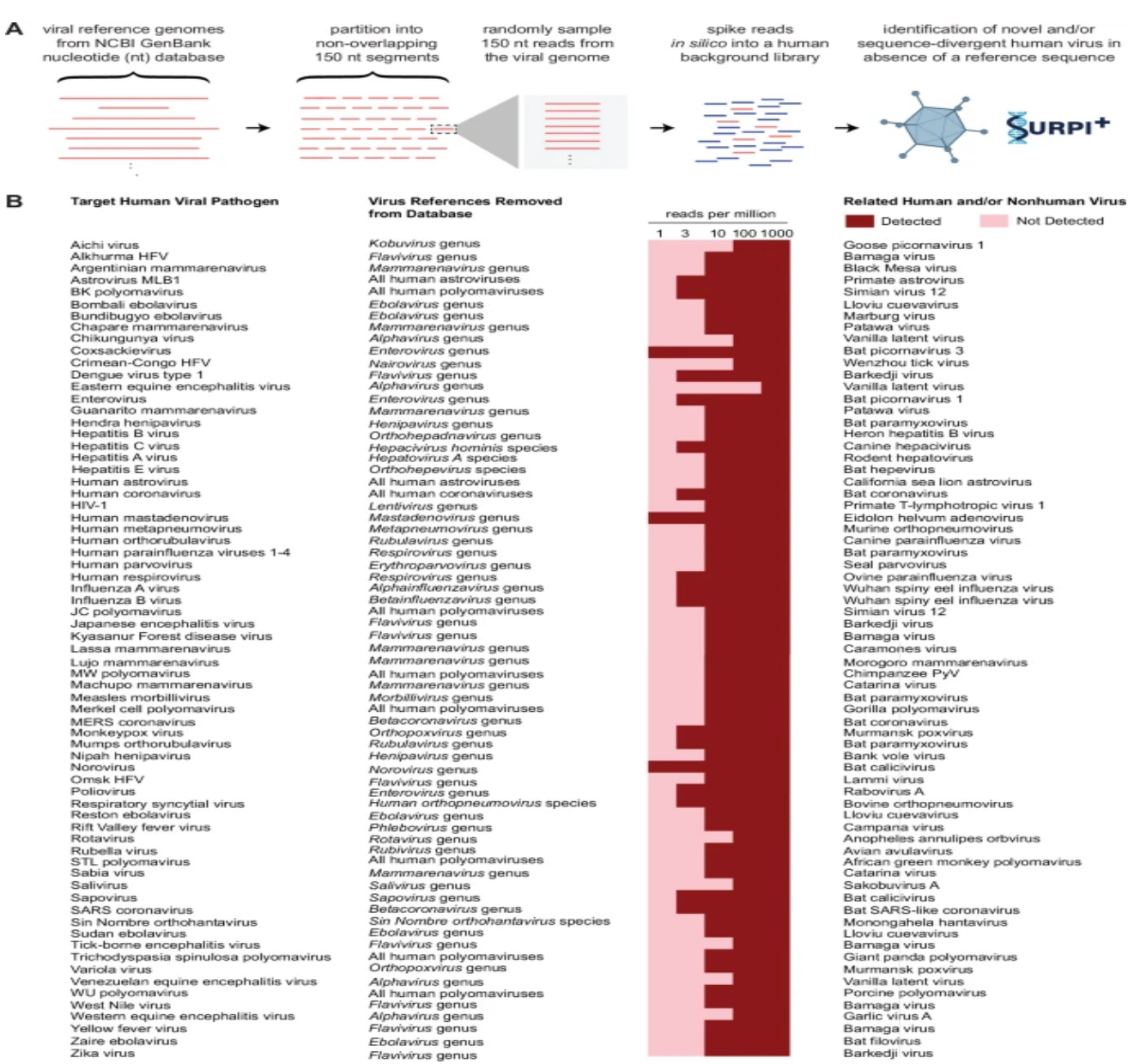
Nat Commun. 2024 Nov 12;15(1):9016. doi: 10.1038/s41467-024-51470-y.

Laboratory validation of a clinical metagenomic next-generation sequencing assay for respiratory virus detection and discovery

Jessica Karielle Tan [# 1 2], Venice Servellita [# 1 2], Doug Stryke [# 1 2], Emily Kelly [1], Jessica Streithorst [1], Nanami Sumimoto [1 2], Abiodun Foresythe [1 2], Hee Jae Huh [1 2 3], Jenny Nguyen [1 2], Miriam Oseguera [1 2], Noah Brazer [1 2], Jack Tang [1 2], Danielle Ingebrigtsen [1], Becky Fung [1], Helen Reyes [1], Melissa Hillberg [1], Alice Chen [4], Hugo Guevara [4], Shigeo Yagi [4], Christina Morales [4], Debra A Wadford [4], Peter M Mourani [5], Charles R Langelier [6 7], Mikael de Lorenzi-Tognon [1 2], Patrick Benoit [1 2], Charles Y Chiu [8 9 10 11]

Fig. 2: Enhancements to the SURPI+ bioinformatics pipeline for pathogen identification.





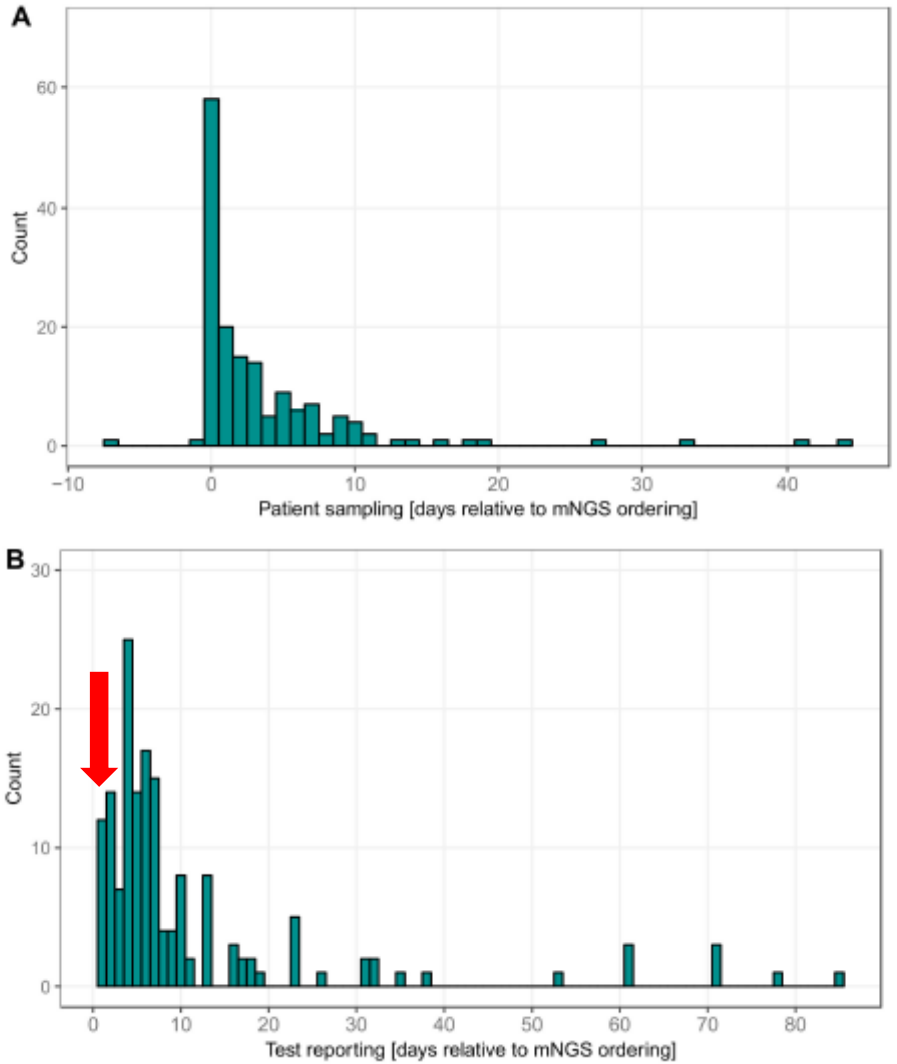
Article

Two Years of Viral Metagenomics in a Tertiary Diagnostics Unit: Evaluation of the First 105 Cases

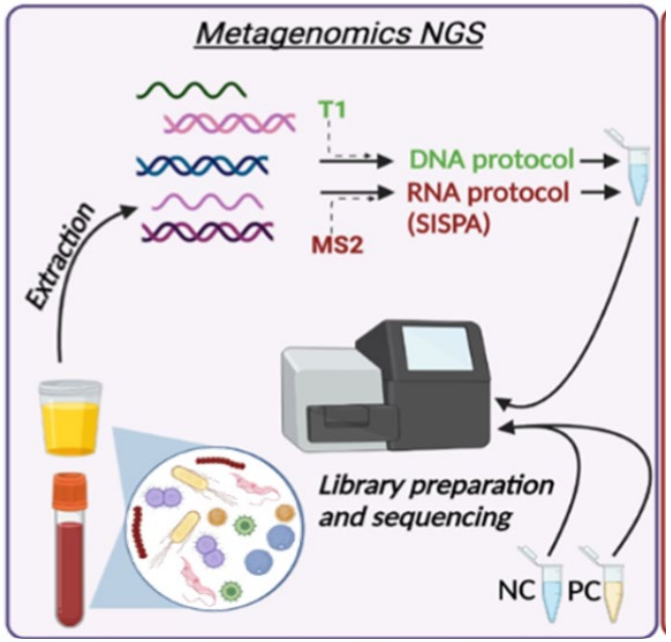
Verena Kufner [1,†], Andreas Plate [2,†], Stefan Schmutz [1], Dominique L. Braun [1,2], Huldrych F. Günthard [1,2], Riccarda Capaul [1], Andrea Zbinden [1], Nicolas J. Mueller [2,*], Alexandra Trkola [1,*] and Michael Huber [1,*]

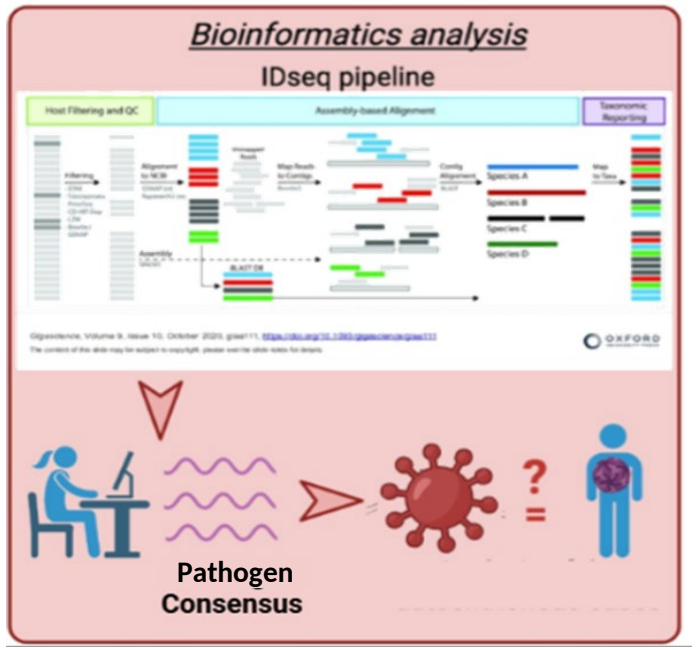
					Respective Conventional Testing	
					+	−
All Samples	OPA = 81/94 [1]	PPA = 65/92% [1]	mNGS	+	22	2
		NPA = 95%		-	2 pos 10 low pos [1]	39
CSF	OPA = 81/91% [2]	PPA = 64/88% [2]	mNGS	+	7	1
		NPA = 93%		-	1 pos 3 low pos [2]	14
Blood	OPA = 68/100% [3]	PPA = 46/100% [3]	mNGS	+	5	0
		NPA = 100%		-	0 6 low pos [3]	8
Throat swab	OPA = 91%	PPA = 100%	mNGS	+	4	1
		NPA = 86%		-	0	6

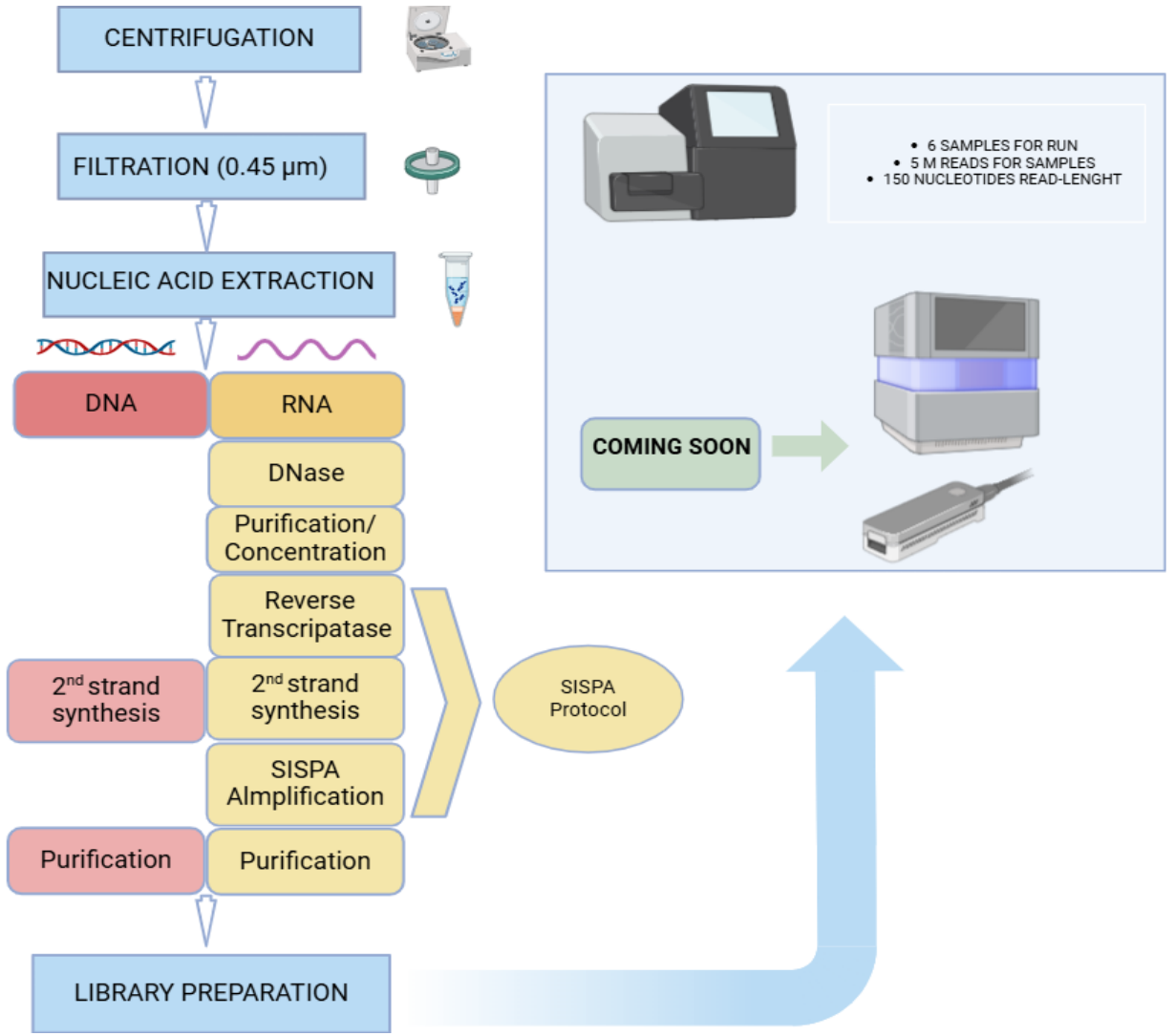
We next looked at the cases with a result reported positive by mNGS that were excluded from the analysis above because no respective conventional test was performed. If we excluded viruses that are considered body flora (Anelloviruses [30]) or common skin contamination (Papillomaviruses [21,32]), mNGS detected 24 "infections" of 11 different virus species which were not tested for by a respective conventional test: Pegivirus C (7), Human betaherpesvirus 7 (4), Norwalk virus (3), Human immunodeficiency virus 1 (2), Hepatitis B virus (2), Influenza A virus (1), Human alphaherpesvirus 1 (1), Human alphaherpesvirus 2 (1), Hepatitis C virus (1), Betacoronavirus 1 (1), and Aeromonas virus phiO18P (1).



Metagenomics Protocol

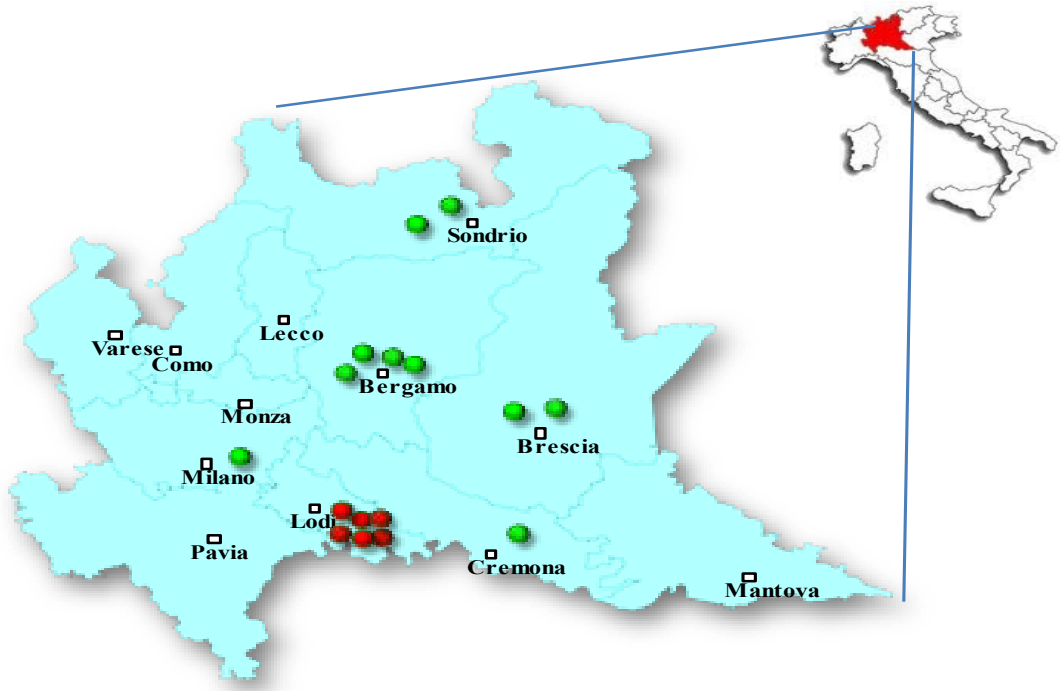






Preliminary results on an autochthonous dengue outbreak in Lombardy Region, Italy, August 2023

Irene Cassaniti [1 2 3], Guglielmo Ferrari [2 3], Sabrina Senatore [4], Eva Rossetti [4], Francesco Defilippo [5], Manuel Maffeo [6 7], Luigi Vezzosi [7 8], Giulia Campanini [3], Antonella Sarasini [3], Stefania Paolucci [3], Antonio Piralla [3], Davide Lelli [5], Ana Moreno [5], Maira Bonini [4], Marcello Tirani [8 9], Lorenzo Cerutti [10], Stefano Paglia [11], Angelo Regazzetti [12], Marco Farioli [8], Antonio Lavazza [5], Marino Faccini [4], Francesca Rovida [1 3], Danilo Cereda [13 8], Fausto Baldanti [1 13 3]; Lombardy Dengue network [14]; Lombardy Dengue Network



Dengue Lombardy outbreak in 2023

Samples

5 x

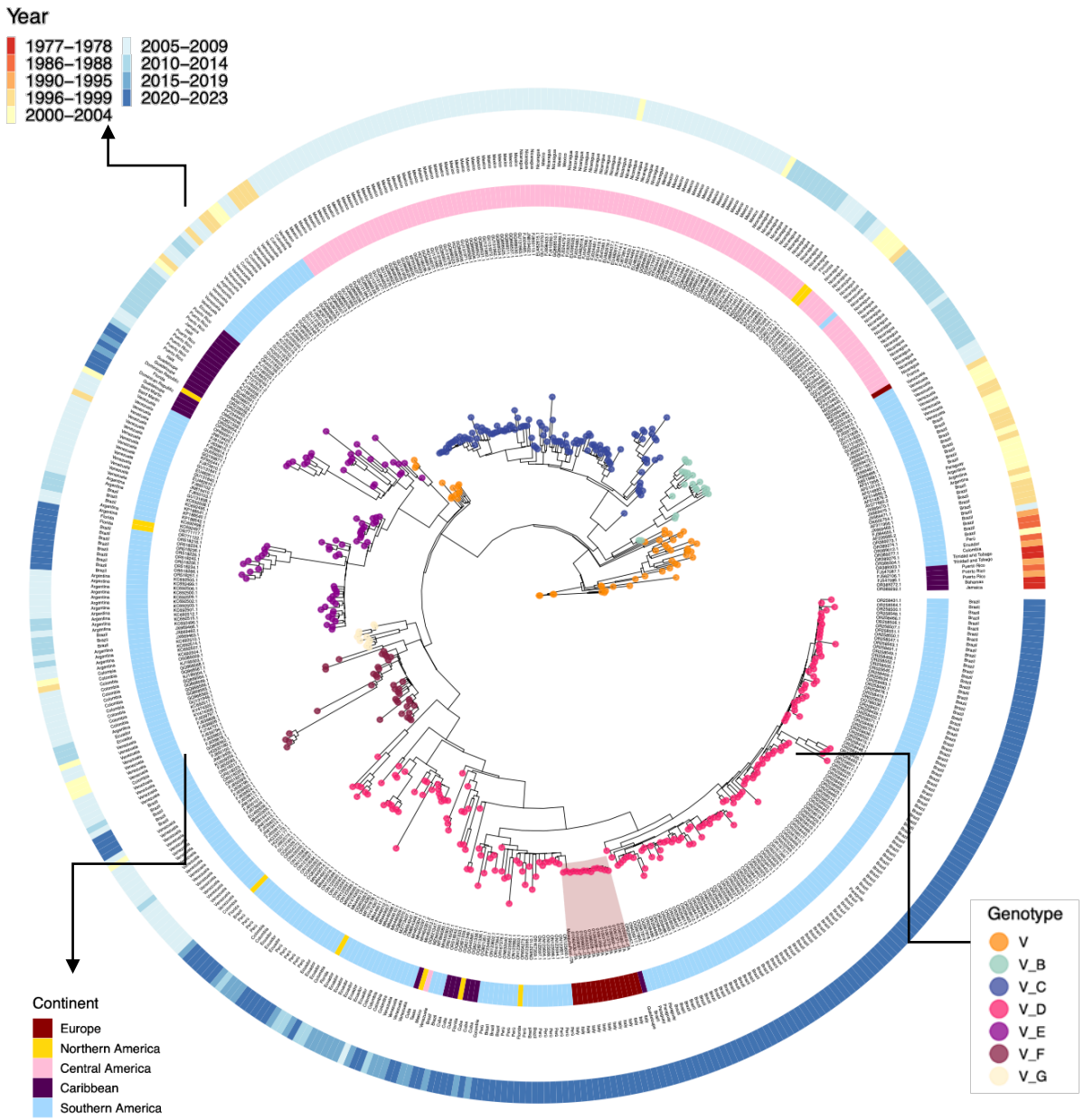
Outbreak

8 x

Screening

1 x

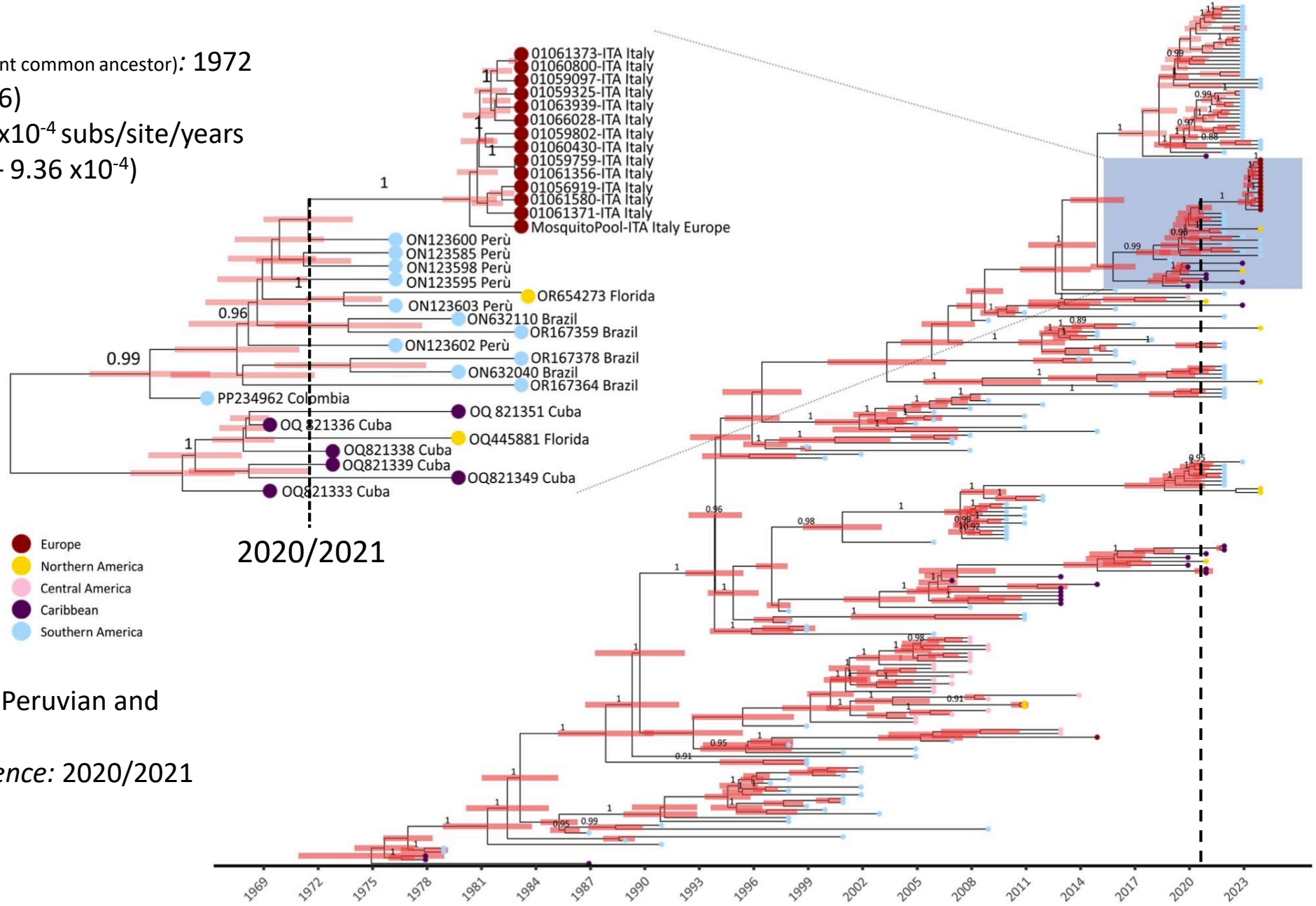
131 *Aedes* (pool)



IQ-TREE v.1.6.8 [B.Q. Minh et al., *Mol. Biol. Evol., 2020*]

Dengue Lombardy outbreak in 2023

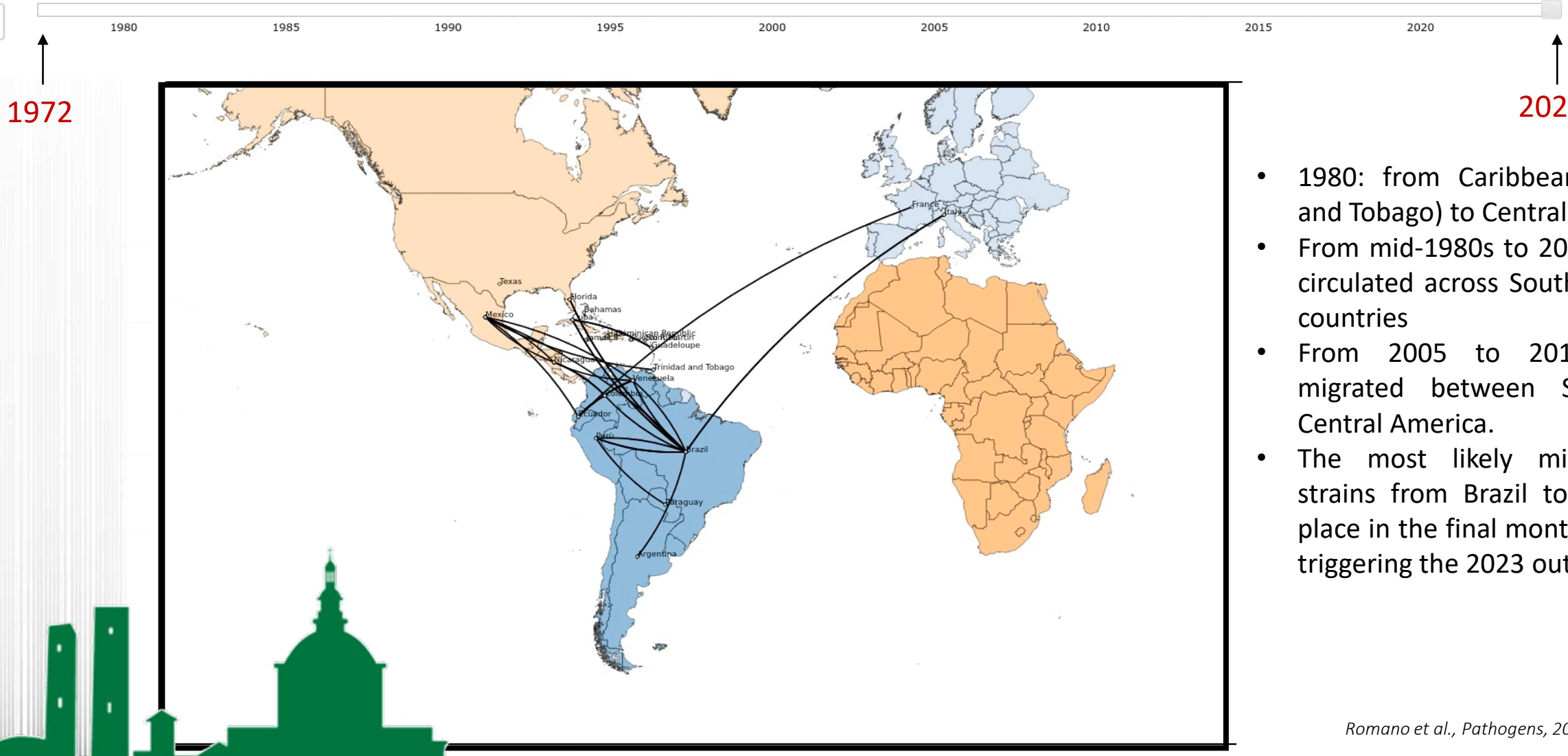
- *Year of tMRCA*(time to the most recent common ancestor)*:* 1972 (95% HPD interval: 1968 - 1976)
- *Mean Substitution Rate:* 8.23 x10^{-4} subs/site/years (95% HPD interval: 7.14 x10^{-4} – 9.36 x10^{-4})



- Italian strains clustered with Peruvian and Brazilian strains
- *Year of Italian strains divergence:* 2020/2021

Romano et al., Pathogens, 2024c

Dengue Lombardy outbreak in 2023



- 1980: from Caribbean (Trinidad and Tobago) to Central America
- From mid-1980s to 2005: strains circulated across South American countries
- From 2005 to 2015: strains migrated between South and Central America.
- The most likely migration of strains from Brazil to Italy took place in the final months of 2022, triggering the 2023 outbreak.

Romano et al., Pathogens, 2024c

Rapid communication

Open Access

Severe and fatal neonatal infections linked to a new variant of echovirus 11, France, July 2022 to April 2023

Like 0

Download

Check for updates

Mathilde Grapin[1,*], Audrey Mirand[2,3,*], Didier Pinquier[4], Aurélie Basset[5], Matthieu Bendavid[1], Maxime Bisseux[2,3], Marion Jeannoël[6], Bérengère Kireche[7], Manoelle Kossorotoff[8], Anne-Sophie L'Honneur[9], Lila Robin[7], Yves Ville[10], Sylvain Renolleau[1], Véronique Lemee[11], Pierre-Henri Jarreau[5], Isabelle Desguerre[8], Florence Lacaille[12], Marianne Leruez-Ville[13], Clémence Guillaume[14], Cécile Henquell[2,3], Alexandre Lapillonne[15], Isabelle Schuffenecker[6,**], Mélodie Aubart[8,16,**]

Between July 2022 and April 2023, nine cases of severe neonatal infection with a liver failure were reported in France. Seven of these children died.

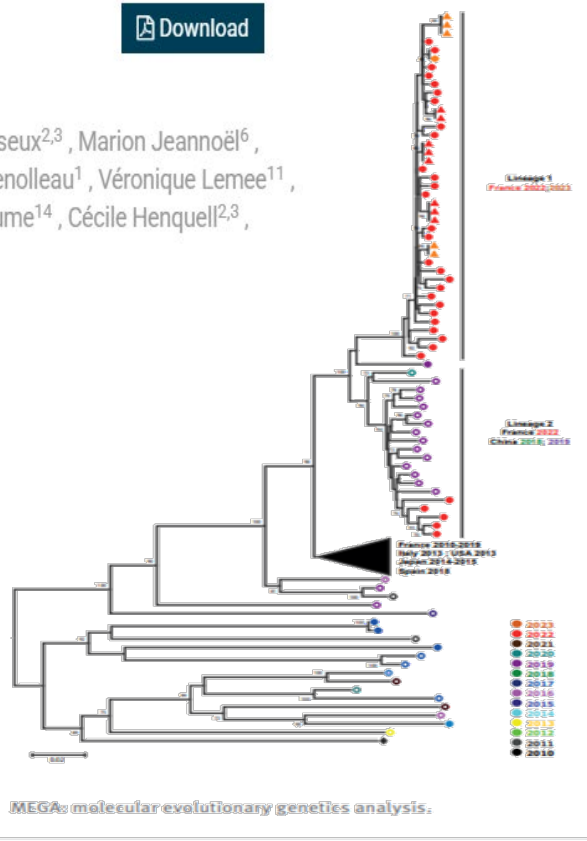


TABLE 2

Virological data on the echovirus-11-infected severe cases and their mothers, France, 2022–2023

Patient	Sample type	Age of the child at sampling (days)	Sequence	Accession number
1	Dried blood spot	3	Not typeable	NA
	CSF	5	Complete 1D^{VP1}	OQ927993
	Stool	6	Complete genome	OQ927998
	Plasma	6	Complete genome	OQ923264
Mother 1	Serum	0	Complete 1D^{VP1}	OQ927994
	Milk	9	Partial 1D^{VP1}	Not deposited
2	Dried blood spot	3	Partial 1D^{VP1}	Not deposited
	Plasma	5	Complete genome	OQ927999
3	Dried blood spot	3	Partial 1D^{VP1}	Not deposited
	Plasma	5	Complete genome	OQ928000
Mother 2–3	Serum	D-3[a]	Complete 1D^{VP1}	OQ927995
4	Dried blood spot	3	Partial 1D^{VP1}	Not deposited
	Psoas muscle biopsy	6	Complete 1^{VP1}	OQ927996
	Liver biopsy	6	Complete genome	OQ928001
	Lung biopsy	6	Complete genome	OQ928002
5	Dried blood spot	2	Partial 1D^{VP1}	Not deposited
	Blood	6	Complete genome	OQ927567
6	CSF	3	Complete genome	OQ928003
7	CSF	3	Complete genome	OQ928004
Mother 6–7	Serum	1	Complete 1D^{VP1}	OQ927997
8	Dried blood spot	3	Partial 1D^{VP1}	Not deposited
	NP swab	8	Partial 1D^{VP1}	Not deposited
	Throat swab	8	Complete genome	OQ969164
	Rectal swab	8	Complete genome	Not deposited (identical to OQ969164)
	Plasma	10	Complete 1D^{VP1}	Not deposited
9	Dried blood spot	3	Partial 1D^{VP1}	Not deposited
	NP swab	8	Partial 1D^{VP1}	Not deposited
	Throat swab	8	Complete genome	OQ969165
	Rectal swab	8	Complete genome	Not deposited (identical to OQ969165)
	Plasma	10	Complete 1D^{VP1}	Not deposited
Mother 8–9	Serum	D-1[a]	Complete 1D^{VP1}	OQ971926

CSF: cerebrospinal fluid; D: delivery; ND: not determined; NP: nasopharyngeal; VP1: capsid protein; 1D: gene coding for VP1 capsid protein.

[a] Samples collected before delivery.

This report suggests that a new variant of E-11 is currently circulating and associated with a high risk of severe neonatal infection and death.

RAPID COMMUNICATION

Fulminant echovirus 11 hepatitis in male non-identical twins in northern Italy, April 2023

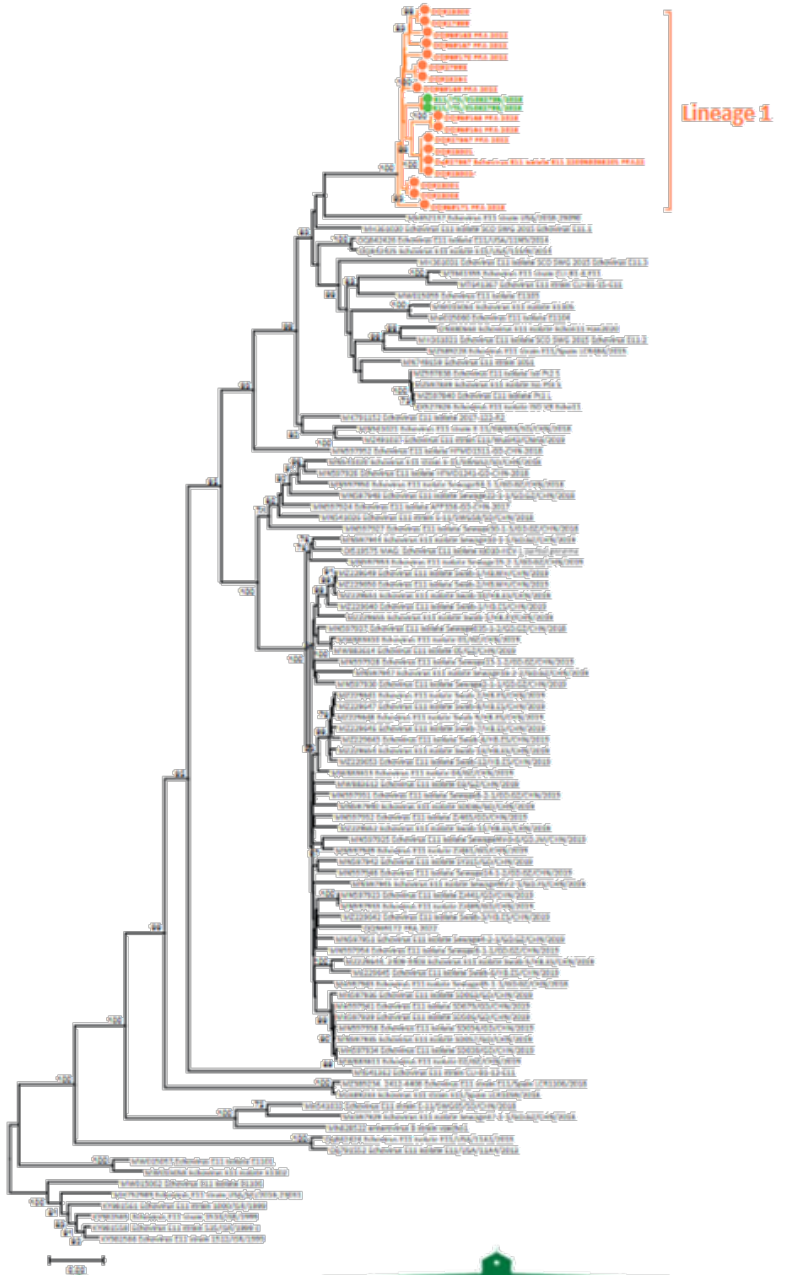
Antonio Piralla[1,*], Alessandro Borghesi[2,*], Amelia Di Comite[2], Federica Giardina[3], Guglielmo Ferrari[1], Simona Zanette[2], Tiziana Angelica Figar[2], Micol Angelini[2], Camilla Pisoni[2], Antonino Maria Guglielmo Pitrolo[1], Stefania Paolucci[1], Francesca Rovida[1,3], Isabella Pellicioli[4], Ezio Bonanomi[4], Fausto Baldanti[1,3,**], Stefano Ghirardello[2,**]

1. Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
2. Neonatal Intensive Care Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
3. Department of Clinical, Surgical, Diagnostic and Paediatric Sciences, University of Pavia, Pavia, Italy
4. Paediatric Intensive Care Unit, Ospedale Papa Giovanni XXIII, Bergamo, Italy
5. These authors contributed equally to this work and share first authorship
6. These authors contributed equally to the work and share the last authorship

Correspondence: Fausto Baldanti (f.baldanti@smatteo.pv.it)

In April 2023, two non-identical, male, late preterm twin brothers, P1 and P2, were transferred from the nursery to the neonatal intensive care unit (NICU) due to episodes of apnoea requiring respiratory support. They were later diagnosed with life-threatening E11 infection.

WGS was performed by mNGS



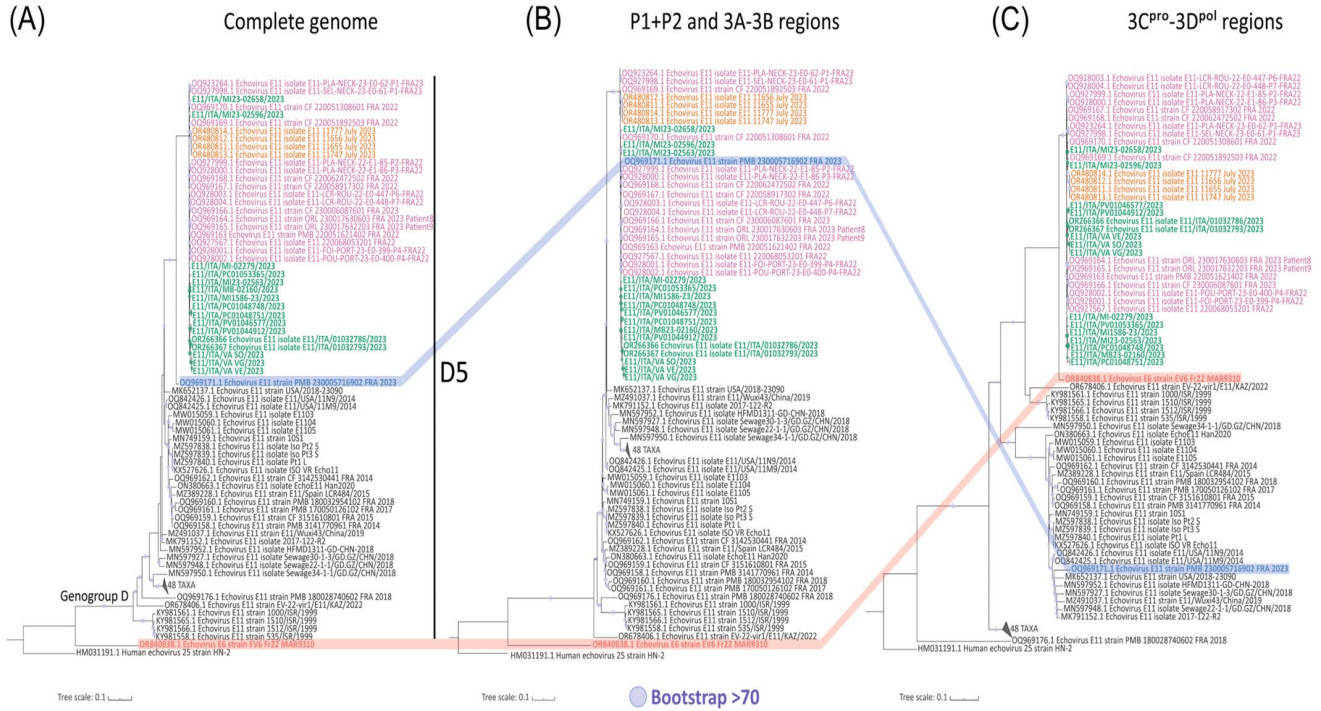
JOURNAL OF MEDICAL VIROLOGY

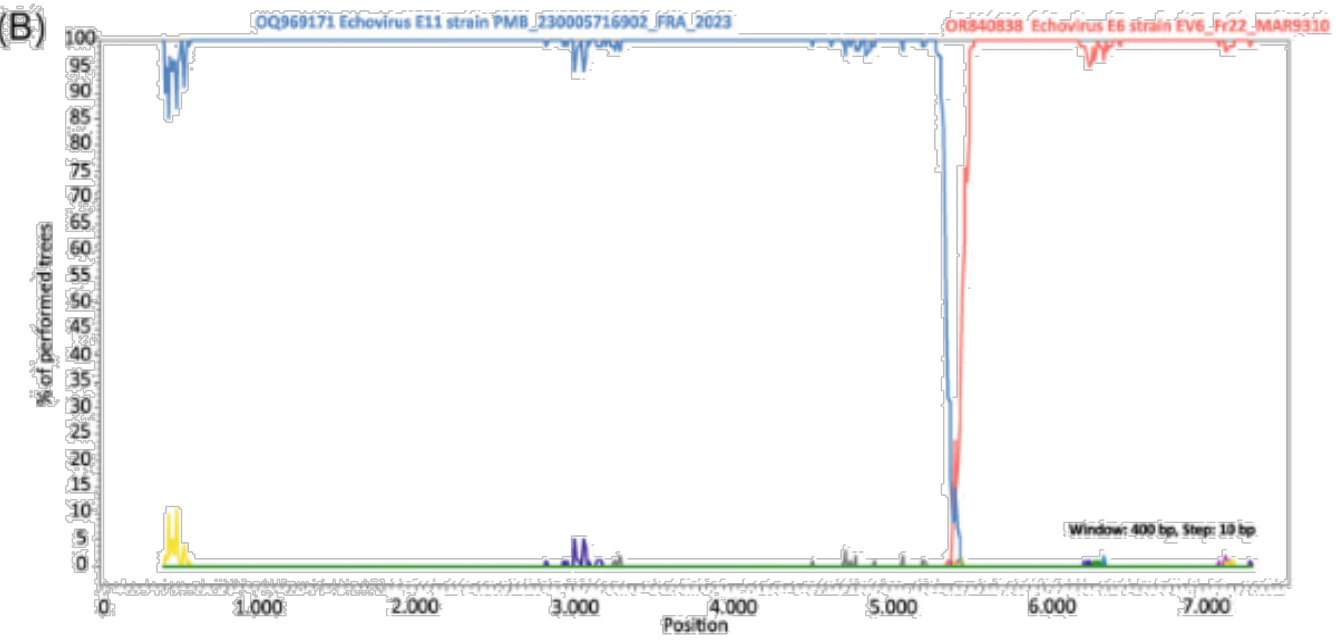
RESEARCH ARTICLE | Open Access

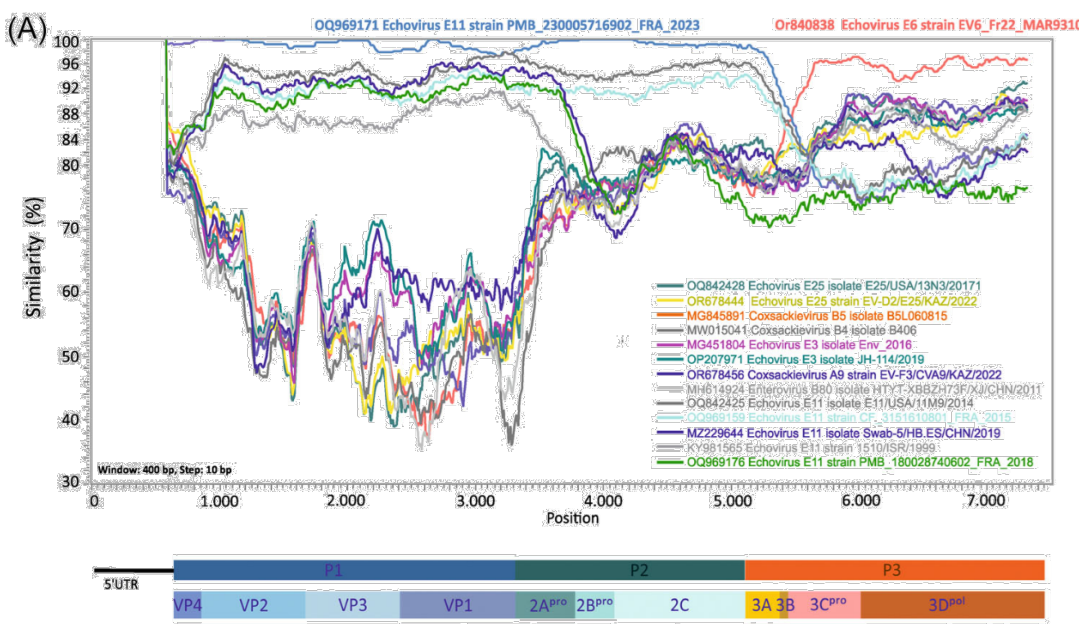
Molecular characterization of emerging Echovirus 11 (E11) shed light on the recombinant origin of a variant associated with severe hepatitis in neonates

Antonio Piralla, Federica Giardina, Guglielmo Ferrari, Stefano Gaiarsa, Greta Romano, Laura Pellegrinelli, Cristina Galli, Arlinda Seiti, Sandro Binda ... See all authors

For both infants, survival was strictly dependent on rapid recognition of the infection and timely administration of intensive care. According to the French report and ours, a host genetic predisposition in male and twin categories might be hypothesised







Centro Nazionale per la Prevenzione ed il Controllo delle Malattie

PROGETTO ESECUTIVO - PROGRAMMA CCM 2022

SURVEID - Studio pilota per la sorveglianza di potenziali minacce da malattie infettive emergenti (EIDs) di origine virale mediante una piattaforma diagnostica basata sul sequenziamento metagenomico di nuova generazione (mNGS).

ENTI PARTECIPANTI:

- UO1 - Regione Lombardia – Direzione Generale Welfare (REG_LOB)
- UO2 - Fondazione IRCCS Policlinico San Matteo (OSM)
- UO3 - Università di Siena (UNISI)
- UO4 - Istituto Nazionale per le Malattie Infettive (INMI) “Lazzaro Spallanzani” (INMI)
- UO5 - Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (IZS_AM)
- UO6 - Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna “Bruno Ubertini” (IZS_LER)
- UO7 - Istituto Zooprofilattico Sperimentale del Lazio e della Toscana “M. Aleandri” (IZS_LT)

Centro Nazionale per la Prevenzione ed il Controllo delle Malattie

PROGETTO ESECUTIVO - PROGRAMMA CCM 2022

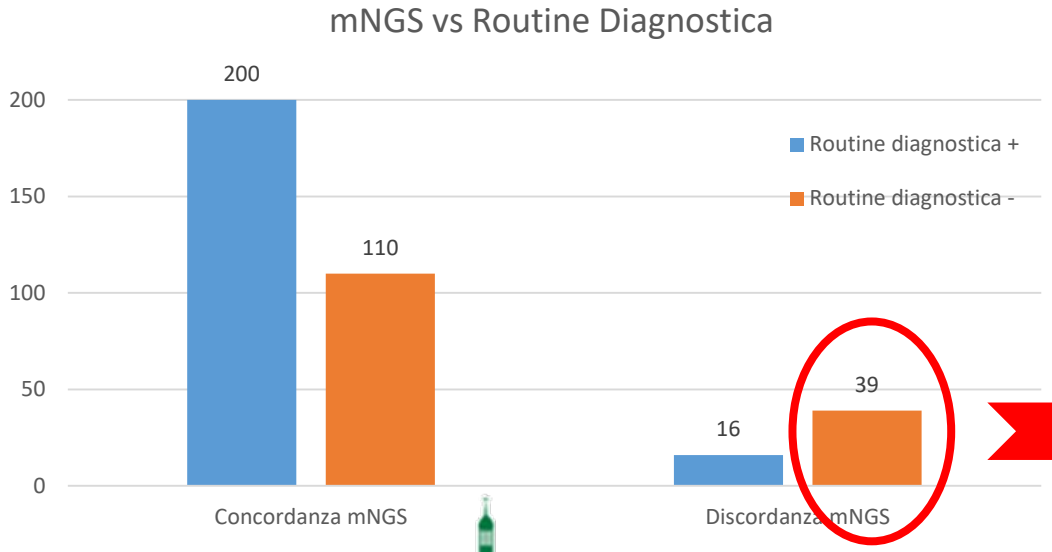
OBIETTIVO GENERALE: Sviluppare una piattaforma diagnostica per identificare nuovi patogeni virali o patogeni riemergenti come possibile causa di minacce per la salute umana e veterinaria mediante sequenziamento metagenomico di nuova generazione (mNGS).

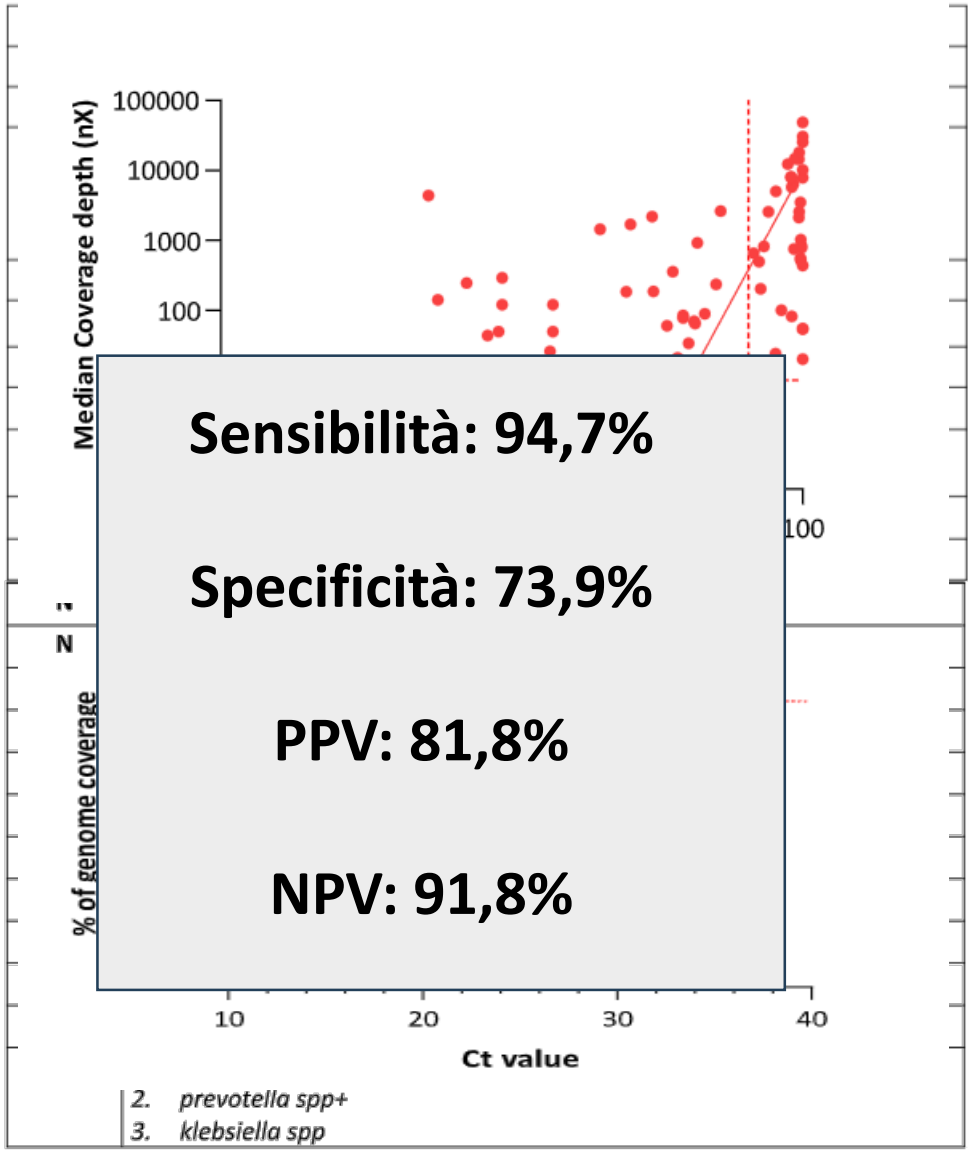
OBIETTIVO SPECIFICO 1: Sviluppo e implementazione di procedure di laboratorio per la mNGS di campioni clinici raccolti da pazienti umani con diverse sindromi cliniche (e.g. neurologiche, respiratorie o sistemiche) e da animali sia selvatici che domestici con quadri patologici anche in corso di focolai ad andamento epidemico, o di animali domestici e selvatici e vettori che fungono di serbatoi di virus zoonotici di importanza per la salute pubblica e comparazione delle performances della mNGS rispetto alle metodiche standard di diagnostica virologica.

OBIETTIVO SPECIFICO 2: Utilizzare la mNGS per il sequenziamento di genomi completi di virus già noti per lo studio delle varianti virali e della presenza di marker molecolari di aumentata patogenicità.

OBIETTIVO SPECIFICO 3: Studio della popolazione virale in diversi distretti corporei mediante raccolta di diversi campioni per l'identificazione di vie di trasmissione alternative e per l'implementazione dei percorsi diagnostici.

	Routine diagnostica +	Routine diagnostica -
Concordanza mNGS	200	110
Discordanza mNGS	16	39





Sensibilità: 94,7%

Specificità: 73,9%

PPV: 81,8%

NPV: 91,8%

Preliminary Data

"So we are talking about a hypothetical disease and to give a name the scientists call it Disease X to prepare for the hypothetical virus or bacteria that in the future can cause large outbreaks or epidemics or pandemics". (WHO-Podcast-Episode #114 - Disease X)

World Health Organization | Health Topics | Countries | Newsroom | Emergencies

Disease Outbreak News

Undiagnose Democratic Congo

8 December 2024

Situation at a glance

Between 24 October and 5 December 2(Republic of the Congo recorded 406 cas headache, cough, runny nose and body malnourished. Among the cases, 31 dea among children, particularly those unde further hindered by the ongoing rainy s 48 hours. These challenges, coupled wit identification of the underlying cause. R of the outbreak and strengthen the resp providing a more detailed clinical charac transmission dynamics, and actively sea the community level. The teams are alsc and community engagement. Given the of associated deaths, acute pneumonia,

Case Reports > Euro Surveill. 2024

doi: 10.2807/1560-7917.ES.2024.29.26.2

Oropouche fever cases diagnosed in Italy in two epidemiologically non-related travellers from Cuba, late May to early June 2024

Concetta Castilletti [1], Antonio Mori [1], Andrea Matucci [1], Niccolò Ronzoni [1], Lukas Van Duffel [2], Giada Rossini [3], Pietro Sponga [1], Maria Luca D'Errico [1], Paola Rodari [1], Francesco Cristini [4 2], Ralph Huits [1], Federico Giovanni Gobbi [5 1]

Affiliations + expand

PMID: 38940002 PMCID: PMC11212459 DOI: 10.2807/1560-7917.ES.2024.29.26.2400362

Editorial > Infect Disord Drug Targets. 2025 Feb 20.

doi: 10.2174/0118715265351833250217061826. Online ahead of print.

Cell

CellPress

Article

Bat-infecting merbecovirus HKU5-CoV lineage 2 can use human ACE2 as a cell entry receptor

Jing Chen,[1,8] Wei Zhang,[2,3,8] Yang Li,[1,8] Chen Liu,[4,8] Tianyi Dong,[1,5] Huiyu Chen,[2,3] Chunguang Wu,[1,5] Jia Su,[1,5] Bei Li,[1] Wei Zhang,[1] Ben Hu,[1] Jingkun Jia,[1,5] Cheng-Bao Ma,[4] Yan Zhu,[1] Xiangyang He,[6] Ang Li,[2,3] Kaiyi Pan,[1,5] Haofeng Lin,[3] Zishuo Guo,[1,5] Cong Li,[7] Libiao Zhang,[6,*] Huan Yan,[4,*] Peng Zhou,[2,3,*] Wei Peng,[2,3,*] and Zheng-Li Shi[2,9,*]

[1]Key Laboratory of Virology and Biosafety, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China
[2]Guangzhou National Laboratory, No. 9 XingDaoHuanBei Road, Guangzhou International Bio Island, Guangzhou, China
[3]The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University, Guangzhou, China
[4]State Key Laboratory of Virology and Biosafety, College of Life Sciences, TaiKang Center for Life and Medical Sciences, Wuhan University, Wuhan, China
[5]University of Chinese Academy of Sciences, Beijing, China
[6]Guangdong Key Laboratory of Animal Conservation and Resource Utilization, Guangdong Public Laboratory of Wild Animal Conservation and Utilization, Institute of Zoology, Guangdong Academy of Sciences, Guangzhou, China
[7]Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, China
[8]These authors contributed equally
[9]Lead contact
*Correspondence: zhanglb@giz.gd.cn (L.Z.), huanyan@whu.edu.cn (H.Y.), zhou_peng@gzlab.ac.cn (P.Z.), peng_wei@gzlab.ac.cn (W.P.), shi_zhengli@gzlab.ac.cn (Z.-L.S.)
https://doi.org/10.1016/j.cell.2025.01.042

...c Health Threat in

...l information

...primo morto per il virus. Cos'è e come si trasmette

di Valeria Pini

Un anziano ha perso la vita a causa dell'Alaskapox che fino a oggi è stato individuato in sette persone

RESEARCH ARTICLE

A Strategy To Estimate Unknown Viral Diversity in Mammals

Simon J. Anthony,[a,b] Jonathan H. Epstein,[b] Kris A. Murray,[b] Isamara Navarrete-Macias,[a] Carlos M. Zambrana-Torrelio,[b] Alexander Solovyov,[a] Rafael Ojeda-Flores,[c] Nicole C. Arrigo,[a] Ariful Islam,[b] Shahneaz Ali Khan,[d] Parviez Hosseini,[b] Tiffany L. Bogich,[e,f] Kevin J. Olival,[b] Maria D. Sanchez-Leon,[a,b] William B. Karesh,[b] Tracey Goldstein,[g] Stephen P. Luby,[h] Stephen S. Morse,[g,i] Jonna A. K. Mazet,[g] Peter Daszak,[b] W. Ian Lipkin[a]

Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, New York, USA[a]; EcoHealth Alliance, New York, New York, USA[b]; Facultad de Medicina Veterinaria and Zootecnia, Universidad Nacional Autónoma de México, Ciudad Universitaria, Mexico City, Distrito Federal, Mexico[c]; Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh[d]; Princeton University, Department of Ecology and Evolutionary Biology, Princeton, New Jersey, USA[e]; Fogarty International Center, National Institutes of Health, Bethesda, Maryland, USA[f]; One Health Institute & Wildlife Health Center, School of Veterinary Medicine, University of California Davis, Davis, California, USA[g]; International Center for Diarrhoeal Disease Research, Dhaka, Bangladesh[h]; Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, New York, USA[i]

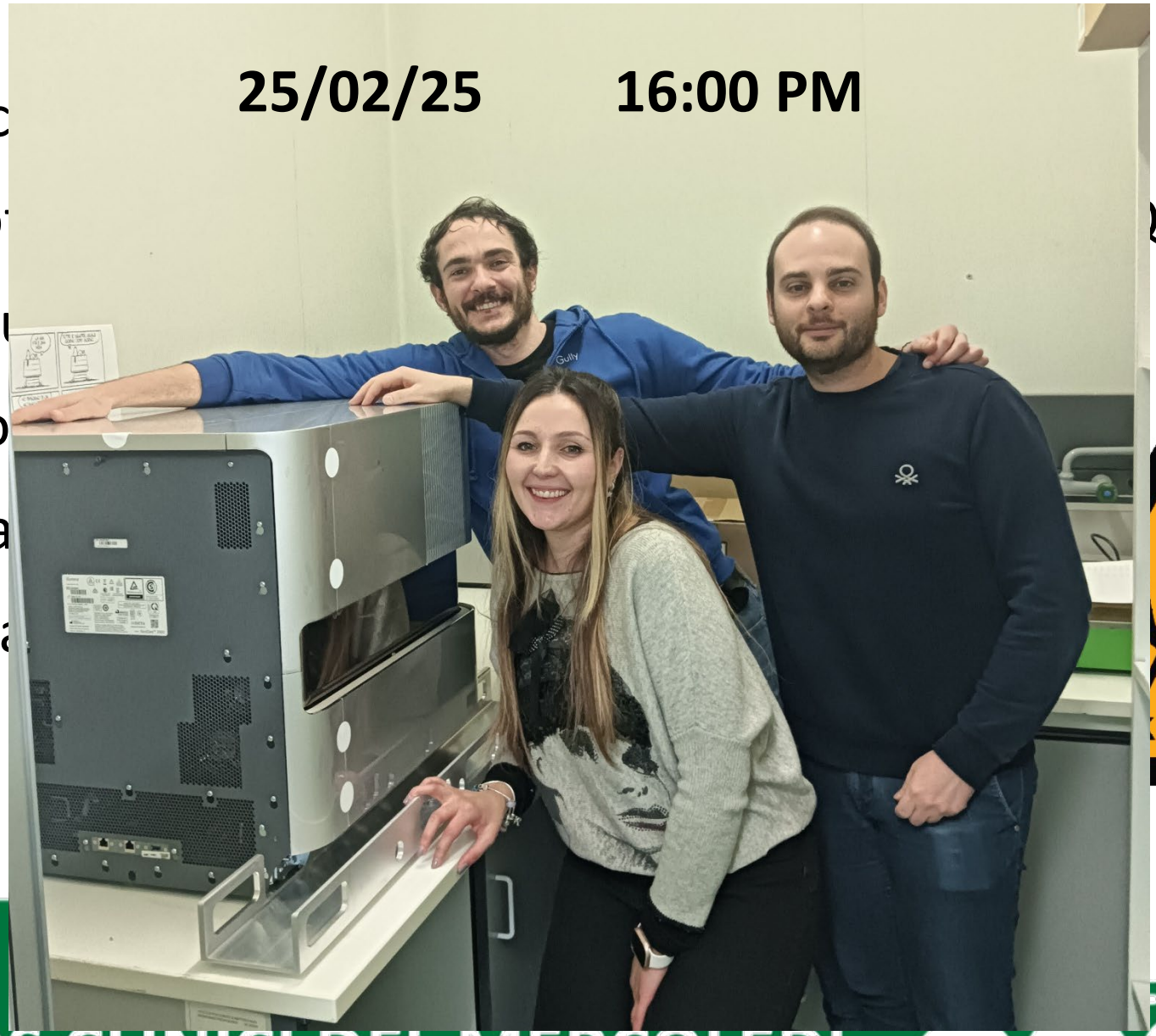
ABSTRACT The majority of emerging zoonoses originate in wildlife, and many are caused by viruses. However, there are no rigorous estimates of total viral diversity (here termed "virodiversity") for any wildlife species, despite the utility of this to future surveillance and control of emerging zoonoses. In this case study, we repeatedly sampled a mammalian wildlife host known to harbor emerging zoonotic pathogens (the Indian Flying Fox, *Pteropus giganteus*) and used PCR with degenerate viral family-level primers to discover and analyze the occurrence patterns of 55 viruses from nine viral families. We then adapted statistical techniques used to estimate biodiversity in vertebrates and plants and estimated the total viral richness of these nine families in *P. giganteus* to be 58 viruses. Our analyses demonstrate proof-of-concept of a strategy for estimating viral richness and provide the first statistically supported estimate of the number of undiscovered viruses in a mammalian host. We used a simple extrapolation to estimate that there are a minimum of 320,000 mammalian viruses awaiting discovery within these nine families, assuming all species harbor a similar number of viruses, with minimal turnover between host species. We estimate the cost of discovering these viruses to be ~$6.3 billion (or ~$1.4 billion for 85% of the total diversity), which if annualized over a 10-year study time frame would represent a small fraction of the cost of many pandemic zoonoses.

IMPORTANCE Recent years have seen a dramatic increase in viral discovery efforts. However, most lack rigorous systematic de-

320.000

INTO THE FUTURE

- Increasing sequec
- Standardization o
- Decrease turnarou
- DNA and RNA pro
- Bioinformatics sta
- Introduction in dia



Thank You For Your Attention.