vitalant Research Institute

Oropouche Virus

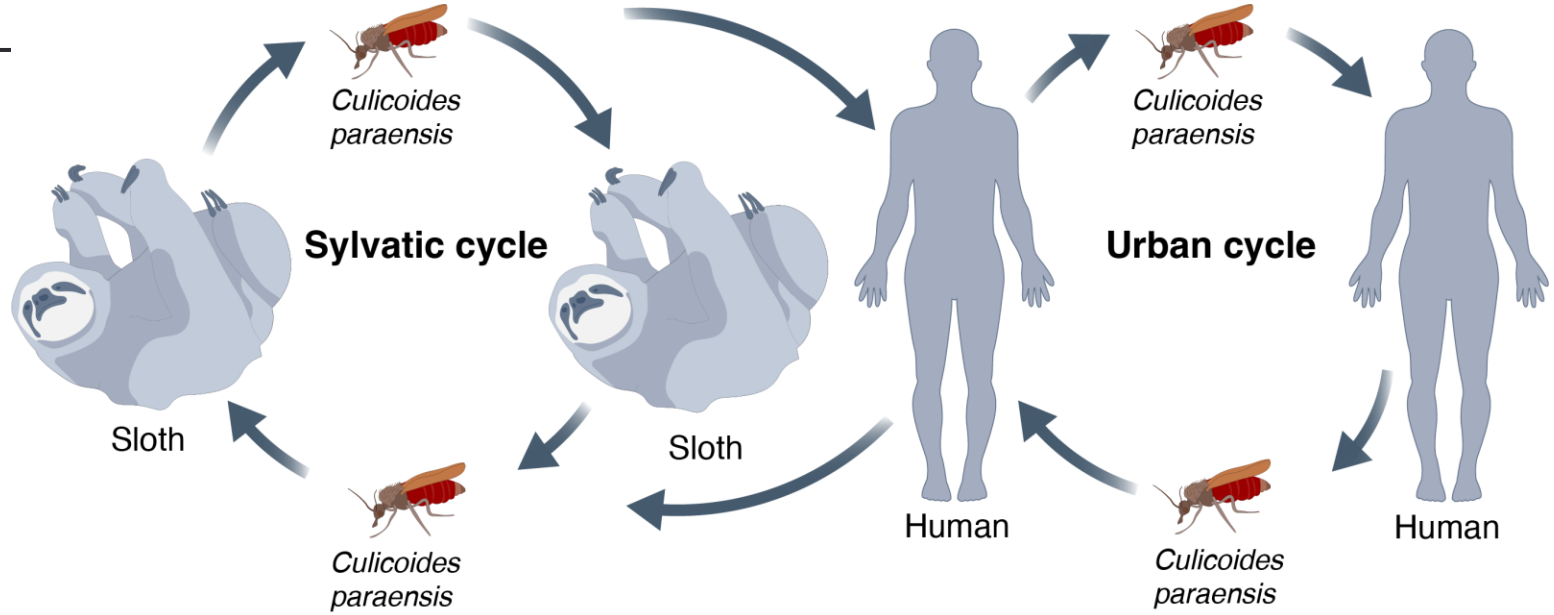
Brian Custer, Pamela Milani, Mike Busch, Ester Sabino

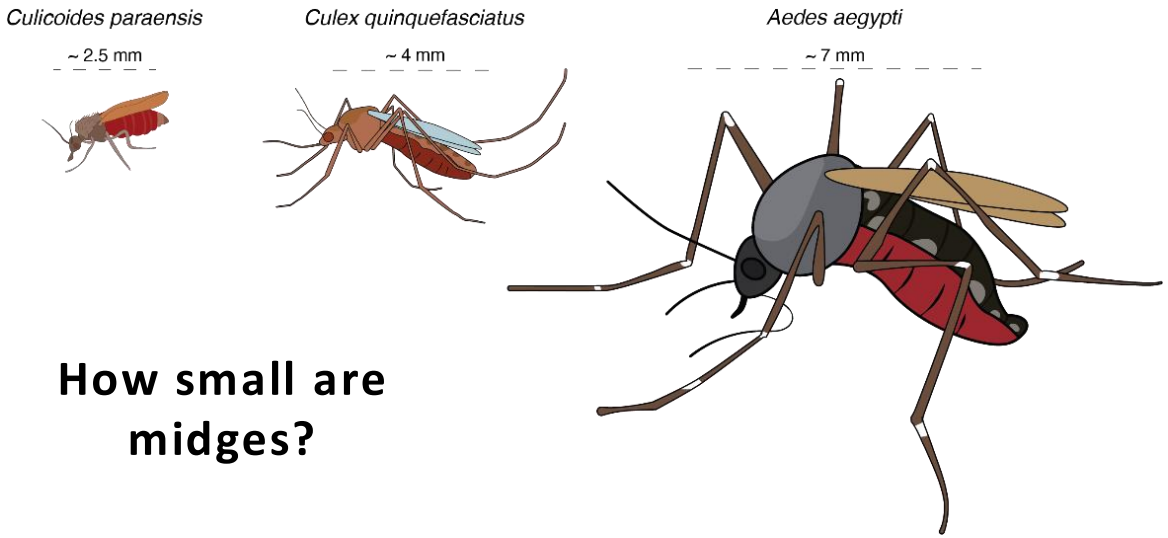
IPFA/PEI 31st International Workshop on Surveillance and Screening of Blood-borne Pathogens

14 - 15 May 2025 | Heidelberg, Germany

Oropouche virus (OROV)

- Neglected tropical disease
- Midge-borne orthobunyavirus
- In Amazon at least since the 1950s
- Febrile illnesses and CNS infection
- Death and pregnancy complications





How small are midges?

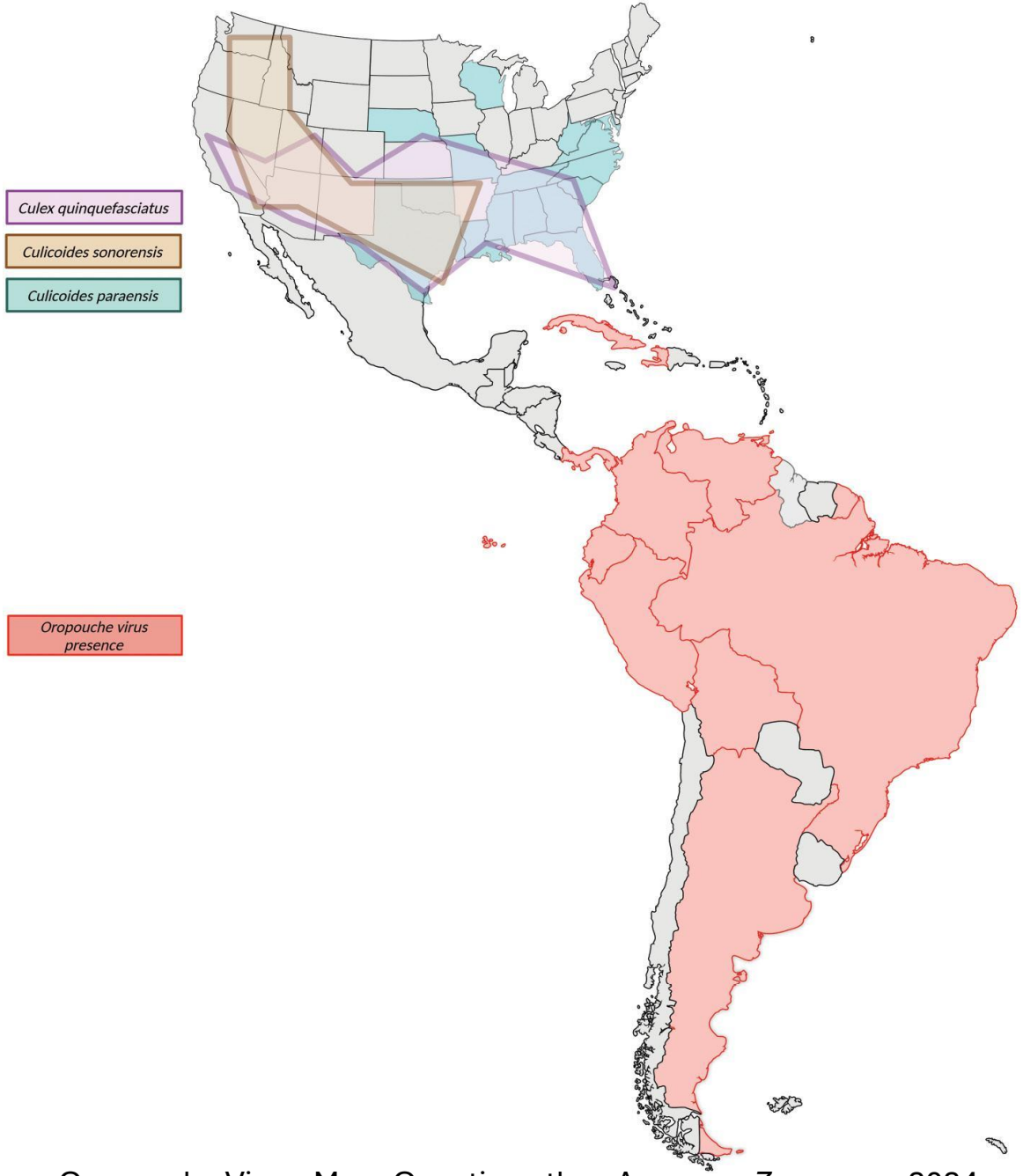


Picture from https://www.paho.org/en/topics/oropouche-virus-disease

Distribution of *Culicoides paraensis?*



Baseline mapping of Oropouche virology, epidemiology, therapeutics, and vaccine research and development March 2022 npj Vaccines 7(1):38 DOI: 10.1038/s41541-022-00456-2



Oropouche Virus: More Questions than Answers. *Zoonoses.* 2024. Vol. 4(1). DOI: 10.15212/ZOONOSES-2024-0006

Oropouche fever

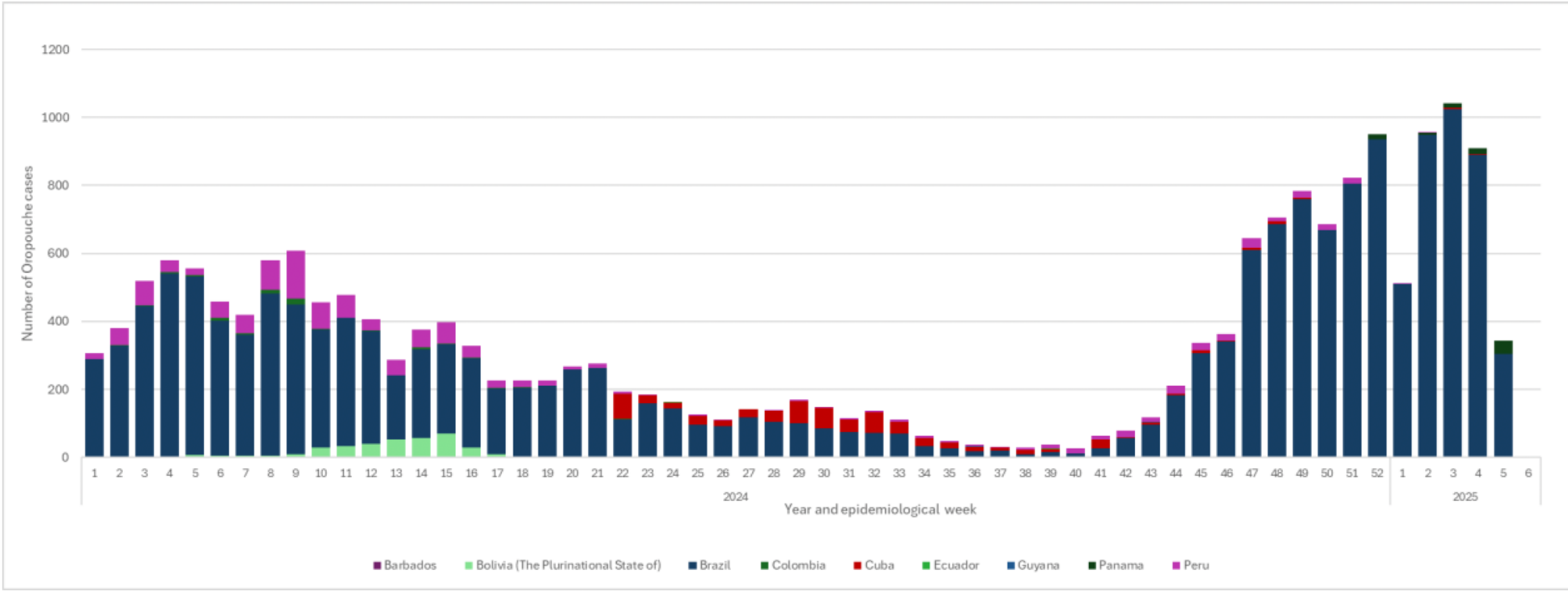
February 2025 PAHO Public Health Risk Assessment:

- In Brazil, there were five confirmed cases of vertical transmission (four fetal deaths and one case of congenital anomaly) and further investigation of 22 fetal deaths, five miscarriages, and four cases of congenital anomaly possibly associated with OROV.

- A similar outbreak occurred in Manaus between 1980 and 1981, with nine infected pregnant women, two of whom miscarried.

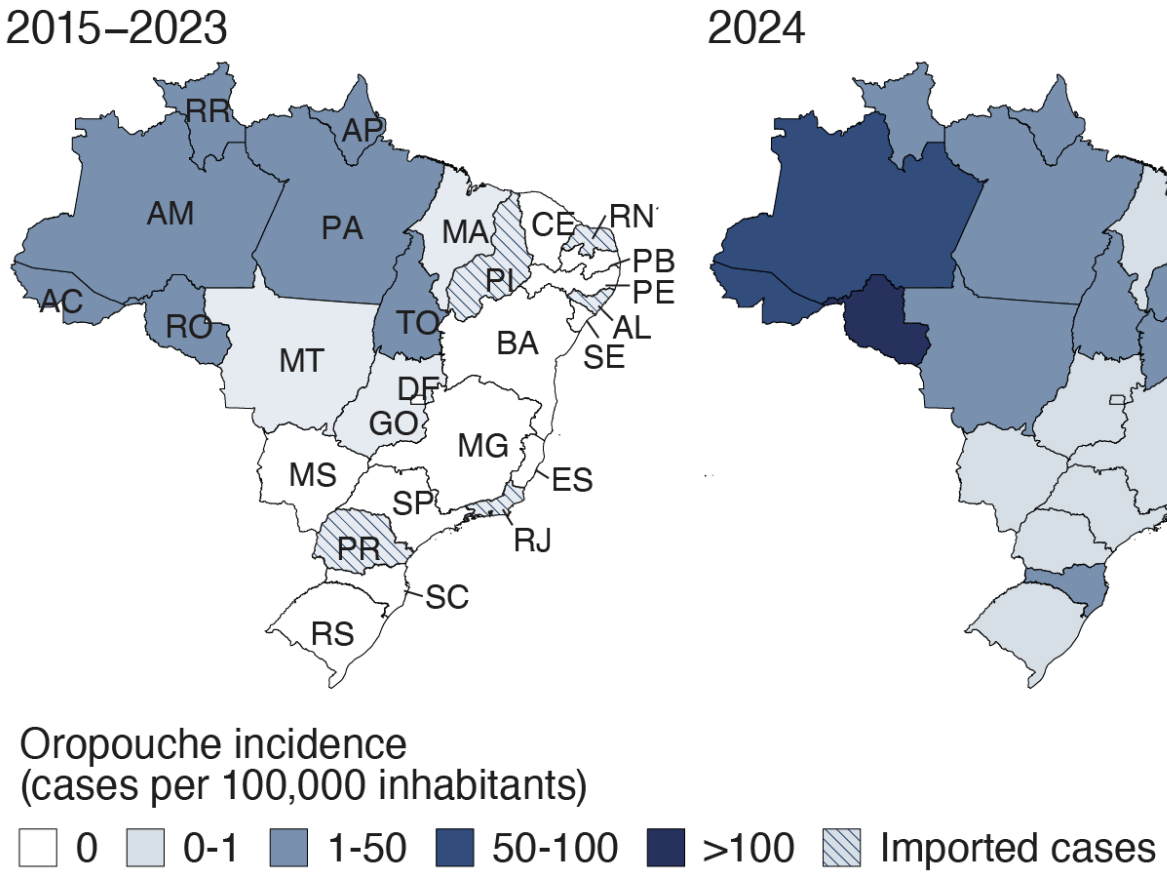
https://www.paho.org/en/documents/epidemiological-alert-oropouche-americas-region-11-february-2025

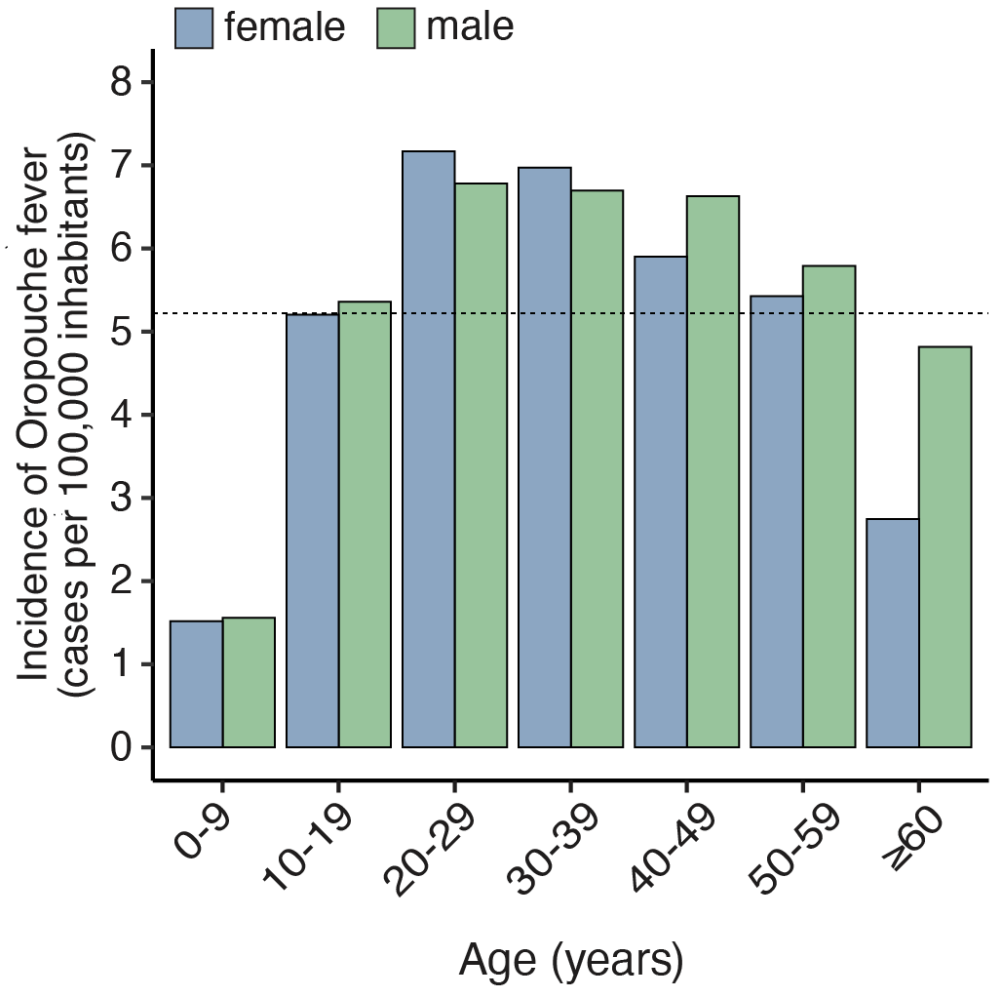
Oropouche in Latin America



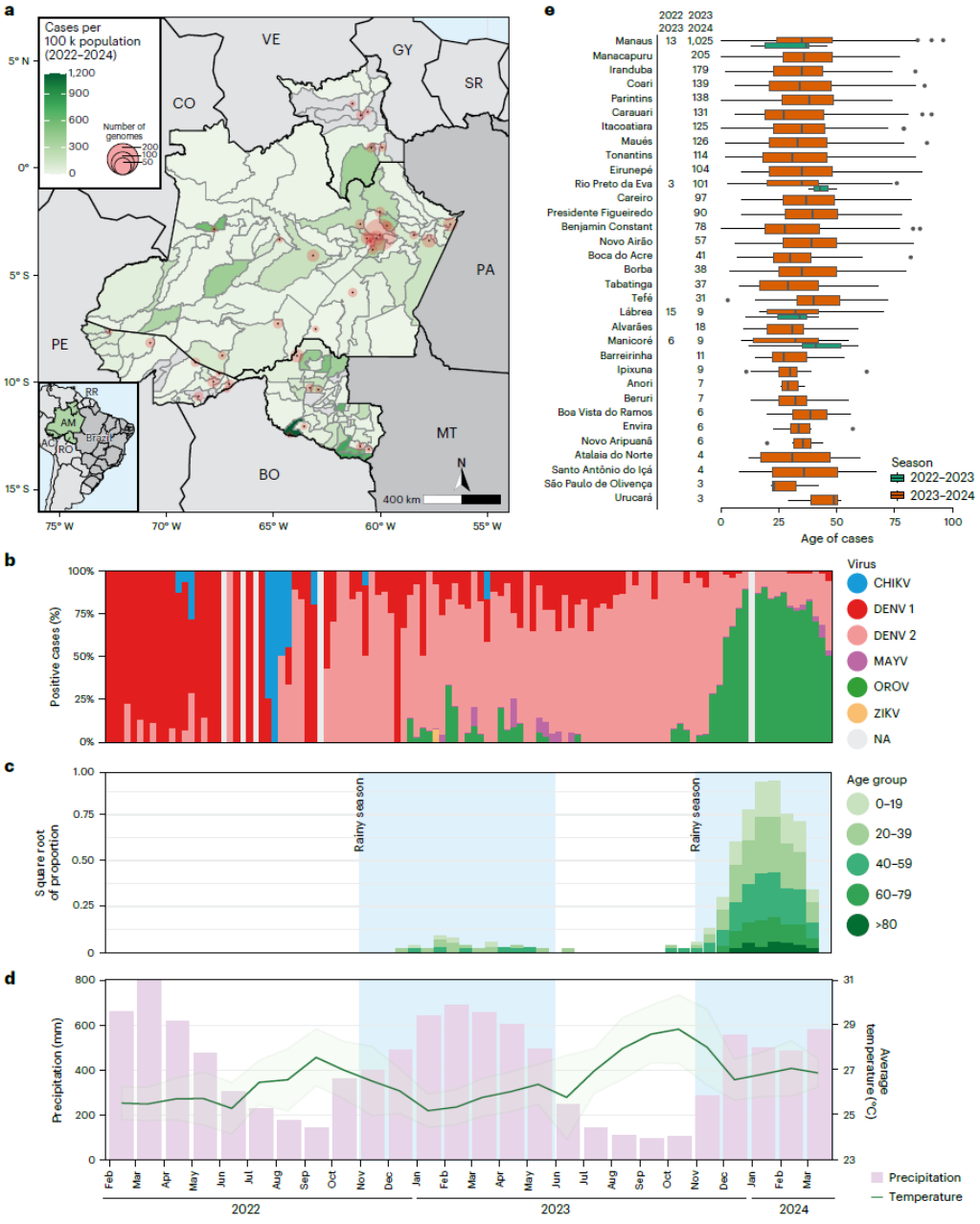
Pan American Health Organization / World Health Organization. Oropouche Epidemiological Update in the Americas Region, 11 February 2025. Washington, D.C.: PAHO/WHO; 2025

Reemergence of Oropouche fever in Brazil





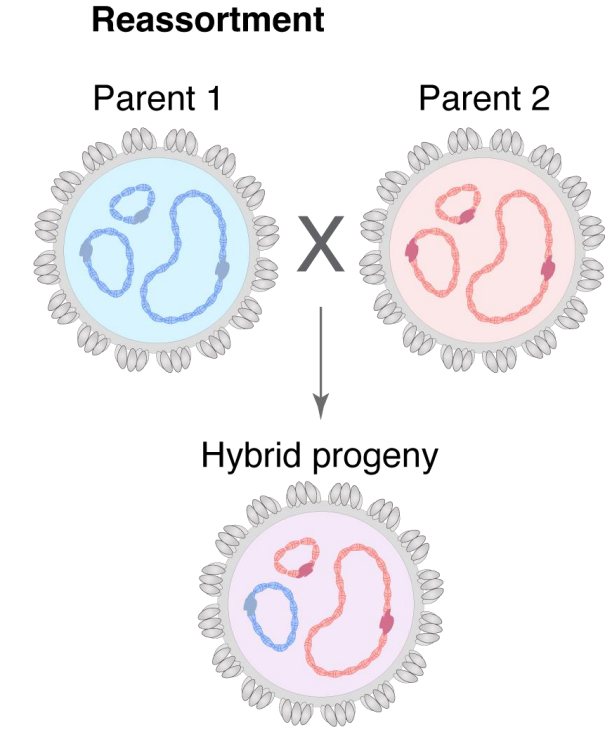
Scachetti et al., the Lancet Infectious Diseases, 2024



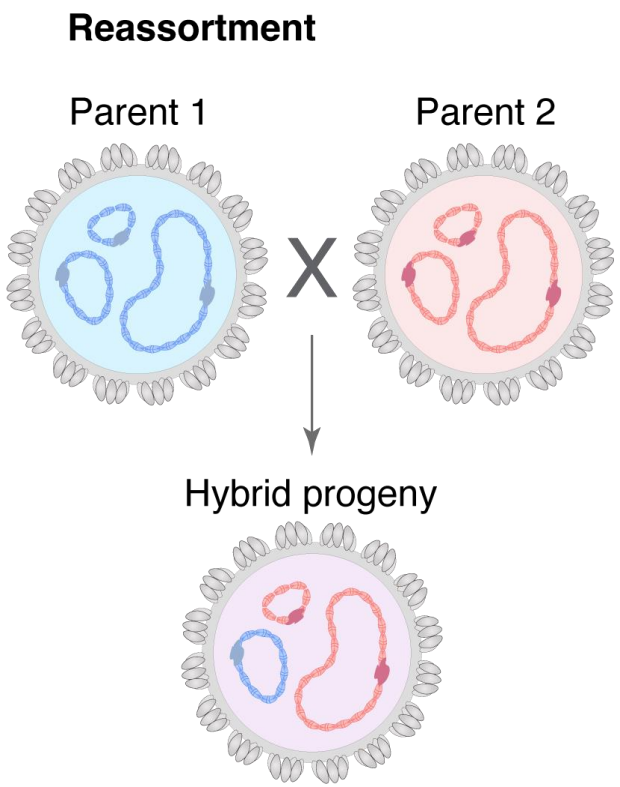
nature medicine

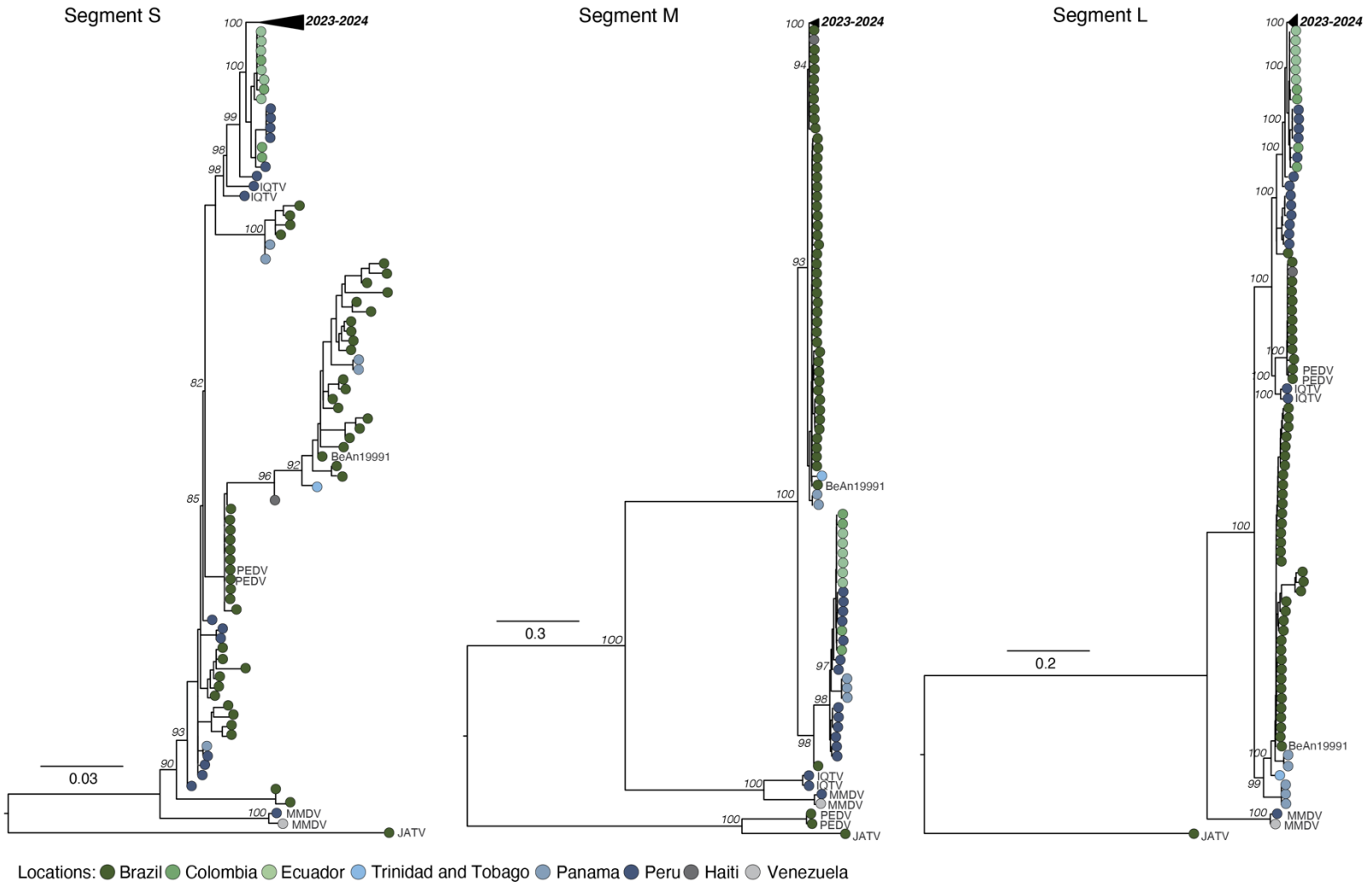
Article https://doi.org/10.1038/s41591-024-03300-3

Human outbreaks of a novel reassortant Oropouche virus in the Brazilian Amazon region



2024 OROV strain is a reassortant

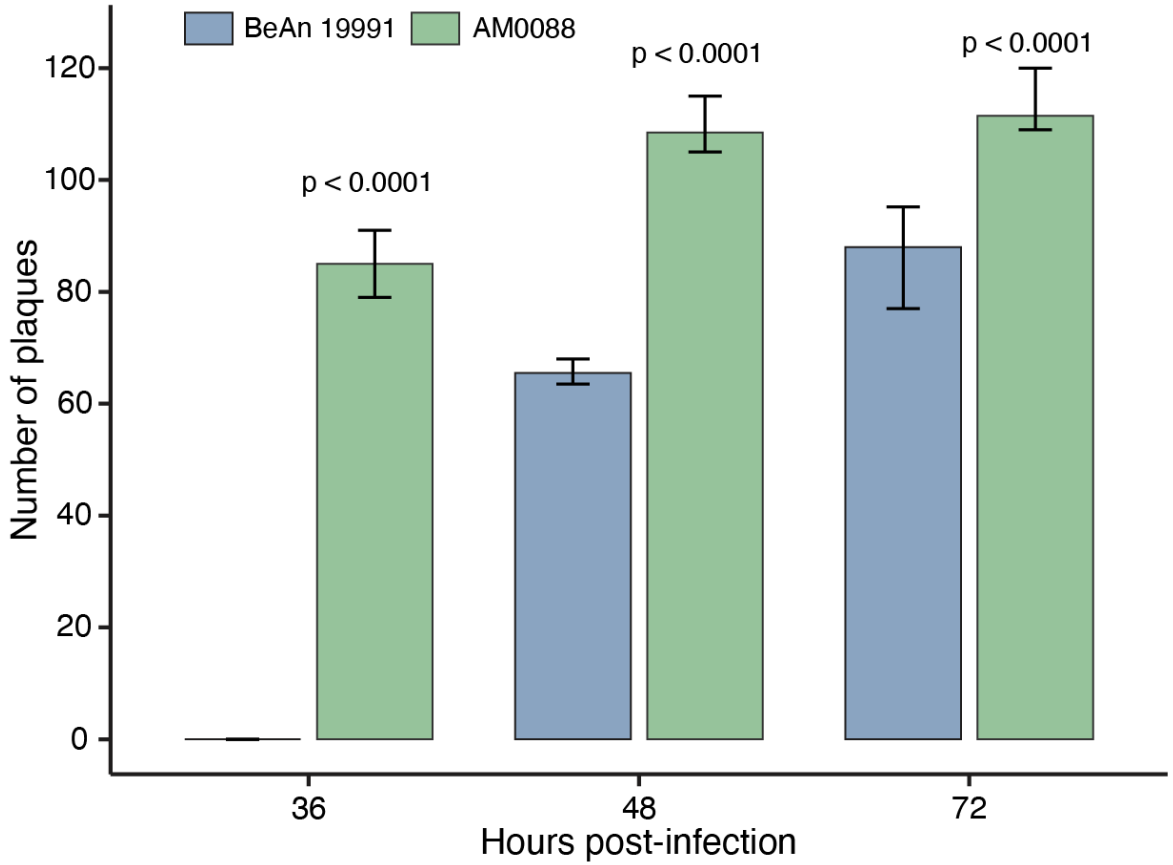


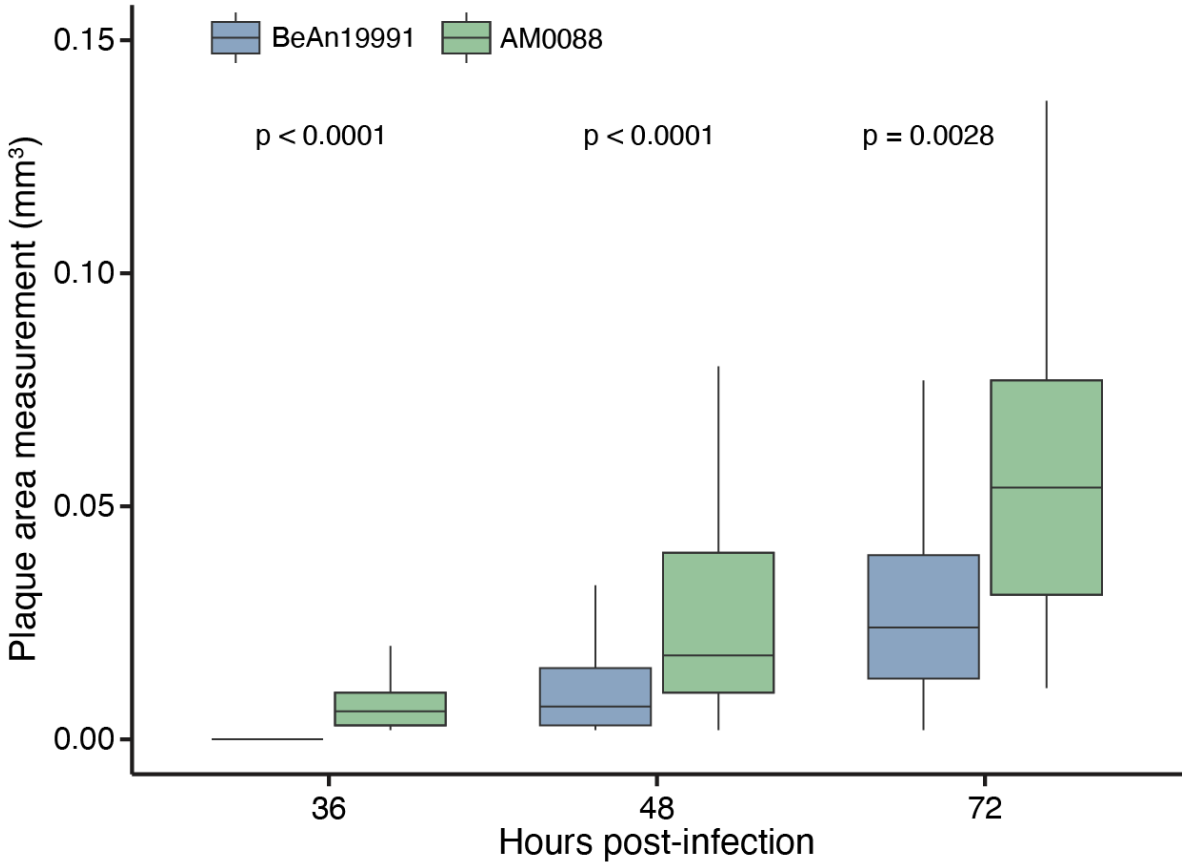


Scachetti et al., the Lancet Infectious Diseases, 2024

New phenotype, viral fitness and potential virulence?

AM0088 has a significantly higher replication at 12 h and 24 h after infection in mammalian cells than the prototype strain





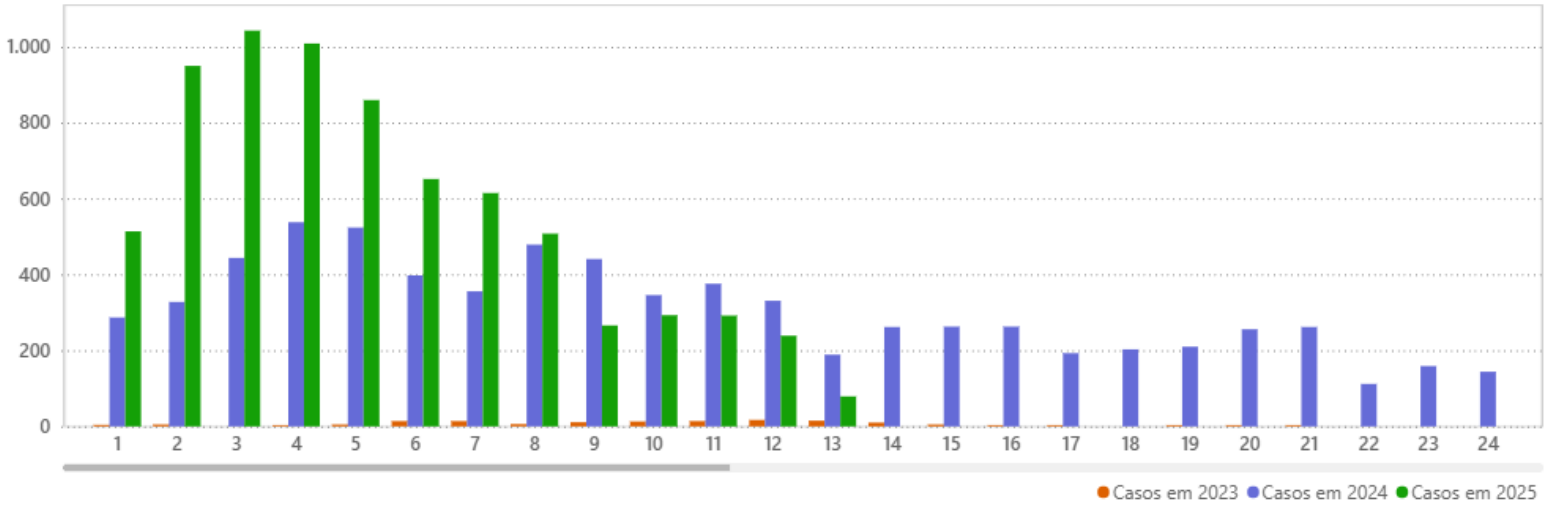
Scachetti et al., the Lancet Infectious Diseases, 2024

Brazil: OROC Case Reports 2023 to 13 week of 2025

vitalant Research Institute

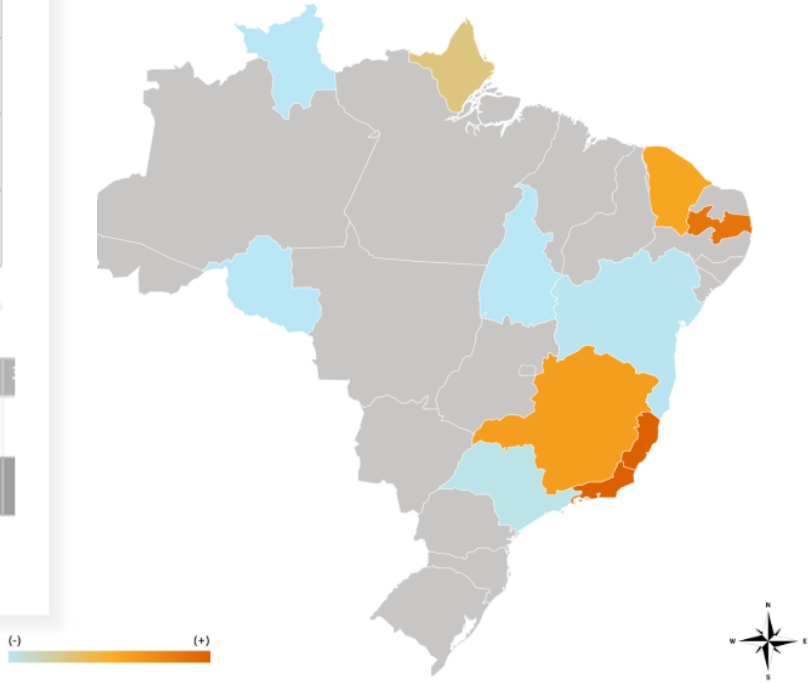
Casos confirmados em 2023	Casos confirmados em 2024	Casos confirmados em 2025	Óbitos em investigação	Óbitos confirmados
832	13.791	7.320	1	0

Número de exames detectáveis para o vírus Oropouche por semana epidemiológica, Brasil



Ano/SE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
2025	514	950	1.043	1.009	860	652	615	508	266	293	292	239	79	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	514	950	1.043	1.009	860	652	615	508	266	293	292	239	79	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Oropouche por UF do Local Provável de Infecção (LPI) e ano, Brasil



Consulte a guia de informações do painel para detalhes sobre a autoctonia em cada UF

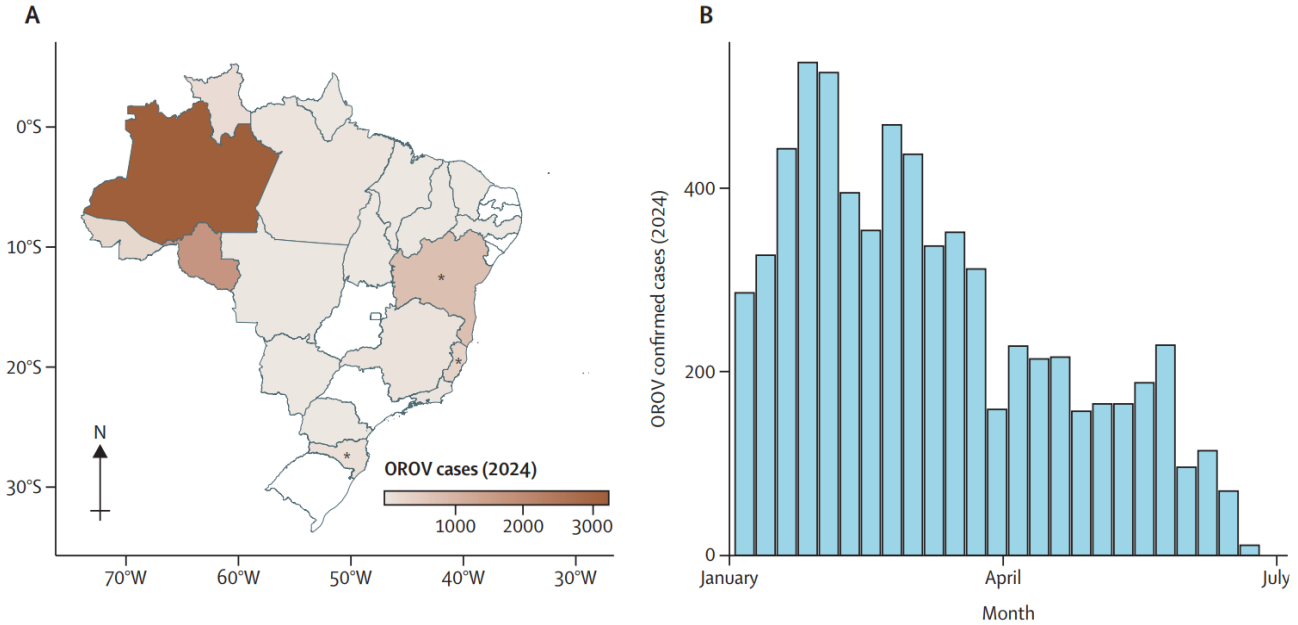
OROV and Blood Centers in Brazil

- No current initiative planning to test donations
- Not aware of testing being developed for blood screening
- Focus on the epidemic response
- Same measures as recommended by US CDC: donor deferral for 28 days following infection

REDS-IV-P Brazil: Process for minipool 18 (MP18) sample collection from Brazil

Collection site: Hemoam (Manaus)

- ~23 MP18 per week created from residual plasma from MP6 testing for HIV, HCV, and HBV
- Collection months of MPs tested: November 2023 – May 2024 (7 months) = 661 minipools already tested for DENV, CHIKV and ZIKV using Grifols RUO triplex prototype assay (all negative for CHIKV and ZIKV, two DENV-positive)
- MP18 testing using VRI inhouse developed OROV assay for period November 2023 – April 2024 (6 months) = 545 MPs consisting of 9,810 donations



https://doi.org/10.1016/ S1473-3099(24)00558-9

Scope of work, assay design specifications and context of use

The (re)emergence of OROV in Brazil in 2023 and 2024 has been dramatic (Guagliardo et al, EID 2024) and may be related to reassortment of the viral genome leading to a more infectious and pathogenic strain (Lancet Infect Dis. 2024 doi: 10.1016/S1473-3099(24)00619-4, and Nat Med. 2024 doi: 10.1038/s41591-024-03300-3). In addition, there is now strong evidence of vertical transmission of OROV and associated fetal demise (Lancet Infect Dis. 2024:S1473-3099(24)00617-0. doi: 10.1016/S1473-3099(24)00617-0, and Viruses. 2024;16(9):1435. doi: 10.3390/v16091435.). These findings have raised concerns about the potential for transfusion-transmission of OROV (Policastro et al. Oropouche virus in Brazil: Assessing the risks and challenges for transfusion medicine; https://doi.org/10.1111/vox.13784).

To characterize rates of OROV viremia, we tested residual volumes following Grifols RUO triplex arbovirus screening from MP of 18 samples from Manaus (Hemoam) from November 2023 through May 2024 using the OROV RT-qPCR assay.

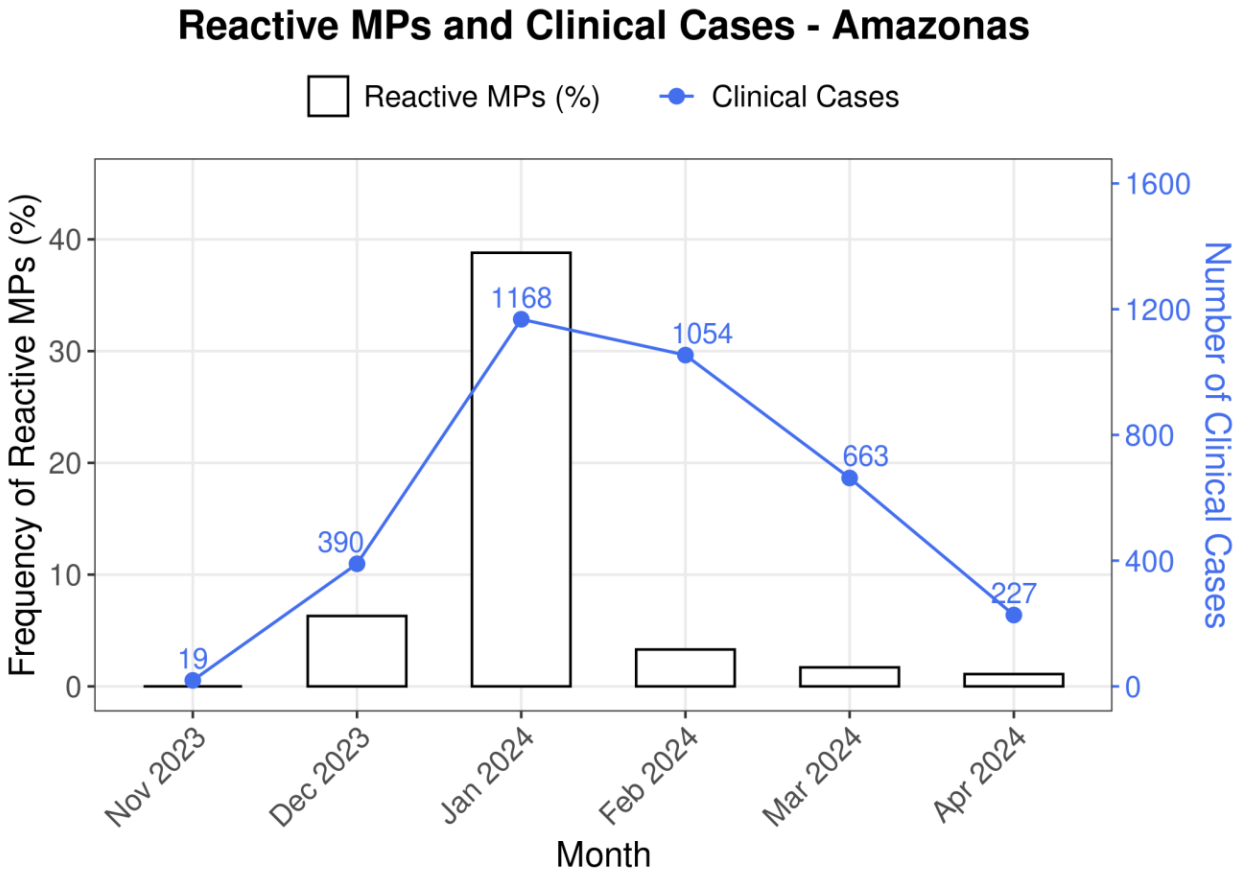
For this assay, viral RNA is extracted from **140 ul** plasma using a commercially available kit (QIAamp Viral RNA Mini Kit) according to the manufacturer's instructions. The RT-qPCR reaction employs primers and a TaqMan probe targeting the S segment of the OROV genome (PMCID: PMC5452489).

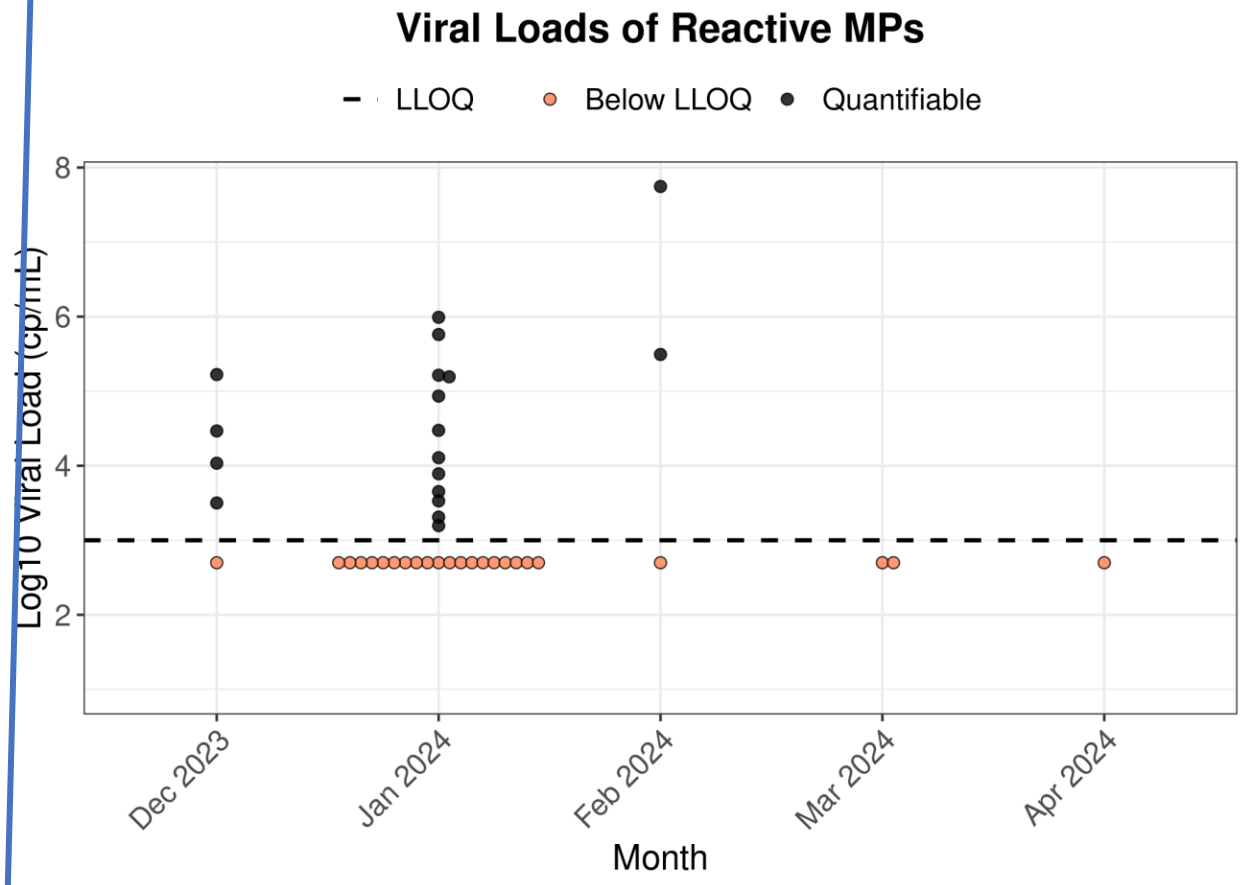
Before proceeding with the testing of the MP18, extensive preliminary was carried out to assess the performance of the assay.

- Naveca et al., Mem Inst Oswaldo Cruz, Vol. 112(7): 510-513, July 2017
- PAHO: https://www.paho.org/sites/default/files/2023-06/mayv-orov-duplexrtpcrprotocolenglish.pdf

LabArchive: OROV qPCR: scope of work and assay design

Results (November 2023 – April 2024)





Eighteen MP samples had viral load values above the LLOQ; their values are shown in the graph.

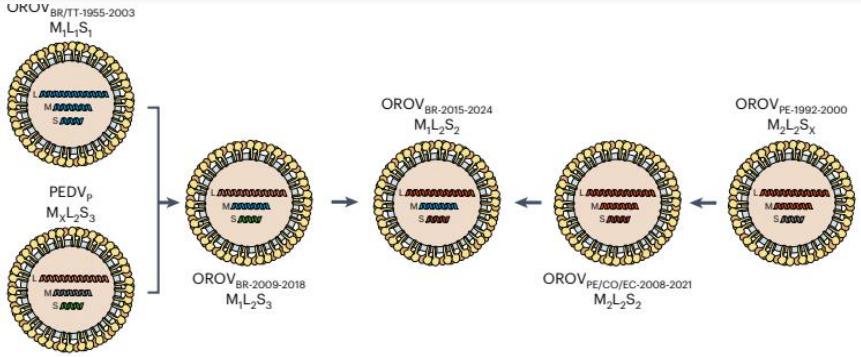
LabArchive: OROV qPCR: REDS-IV-P Brazil MP testing

Next step: Sequence the OROV-positive MPs

vitalant Research Institute

Nat Med. 2024 Dec;30(12):3509-3521. doi: 10.1038/s41591-024-03300-3. Epub 2024 Sep 18.

Human outbreaks of a novel reassortant Oropouche virus in the Brazilian Amazon region



Method: **untargeted** as well as **targeted NGS**, Illumina COVIDseq kit with OROV-specific primers.

Viruses. 2024 Oct 9;16(10):1586. doi: 10.3390/v16101586.

Full Genome Characterization of the First Oropouche Virus Isolate Imported in Europe from Cuba

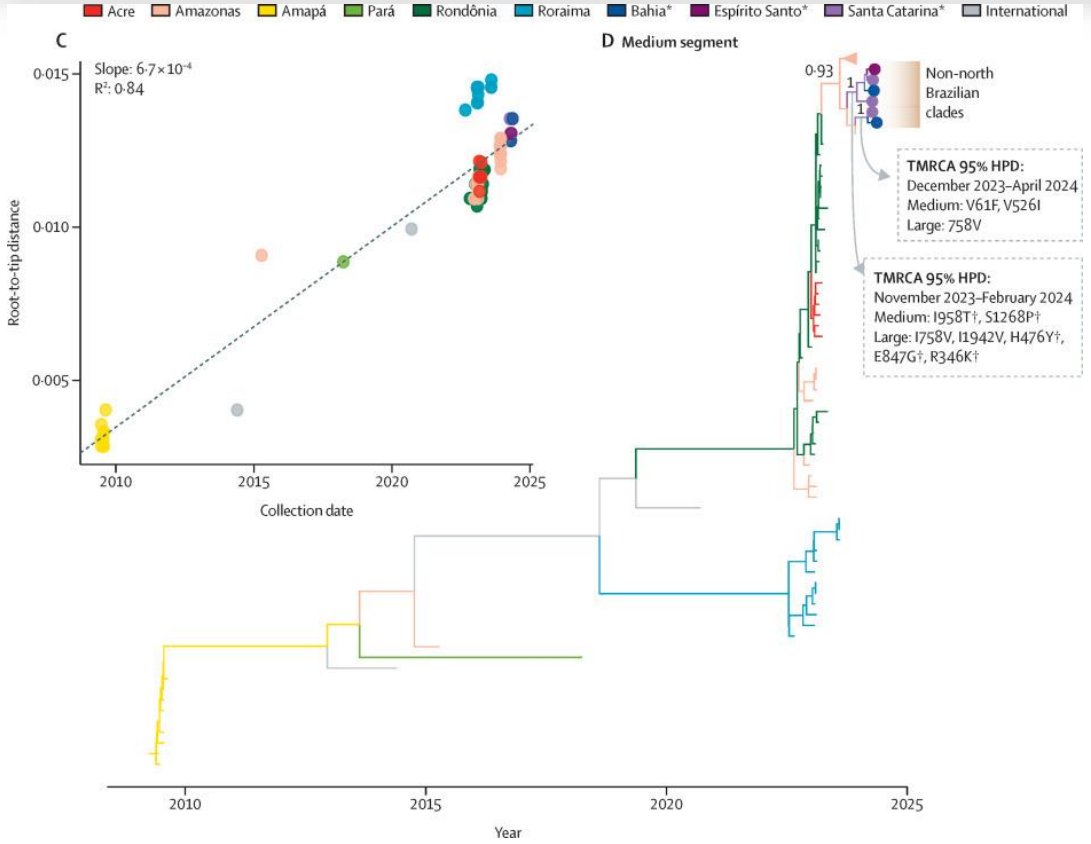
Michela Deiana [1], Simone Malagò [1 2], Antonio Mori [1], Silvia Accordini [1], Andrea Matucci [1], Rebeca Passarelli Mantovani [1], Natasha Gianesini [1], Ralph Huits [1], Chiara Piubelli [1], Federico Giovanni Gobbi [1 3], Maria Rosaria Capobianchi [1], Concetta Castilletti [1]

Extracted RNA was used to generate complete genome sequences using a **metagenomic** approach.
(Untargeted NGS with human rRNA and globin mRNA depletion).

CORRESPONDENCE · Volume 24, Issue 11, E664-E666, November 2024 Download Full Issue

Oropouche virus genomic surveillance in Brazil

Filipe Romero Rebello Moreira [a] · João Victor Rodrigues Dutra [b] · André Henrique Barbosa de Carvalho [c] · Clarisse Rezende Reis [b] · Jéssica Silqueira Hickson Rios [c] · Marisa de Oliveira Ribeiro [d] · et al. Show more



Method: **targeted NGS**, Illumina COVIDseq kit with OROV-specific primers.

Next Steps

- We detected OROV in pooled blood donor samples in Manaus, Brazil

- OROV-positive MP samples are being taken on to NGS to determine the sequences of the infections

- Assessment of seasonal seroincidence in Manaus to define the size of the donor population with evidence of infection in the 2023 – 2024 outbreak season using individual donor samples from Nov ‘23 and May ‘24 using a Luciferase Immunoprecipitation System (LIPS) assay

Current Situation

- The OROV outbreak has expanded to other parts of Brazil and is ongoing
- The vector is found from the tip of South America to mid-continental U.S.
- The viral load of infections from the reassorted OROV is higher than seen in previous strains
- The distribution of OROV is wider than previously thought

(Lancet Infect Dis. 2025 Apr 11:S1473-3099(25)00110-0. doi: 10.1016/S1473-3099(25)00110-0.)

- Imported cases in the US, Canada, Italy, Spain, and Germany are documented

(PAHO Epidemiological Update Oropouche in the Americas Region 11 February 2025)

vitalant℠
Research Institute

Thank you