The spatiotemporal ecology of Oropouche virus across Latin America: a multidisciplinary, laboratory-based, modelling study

Carlo Fischer, Anna Frühauf*, Lucia Inchauste*, Murilo Henrique Anzolini Cassiano, Heriberto Arévalo Ramirez, Karine Barthélémy, Lissete Bautista Machicado, Fernando Augusto Bozza, Carlos Brites, Miguel Mauricio Cabada, César A Cabezas Sánchez, Angie Cervantes Rodríguez, Xavier de Lamballerie, Roxana de los Milagros Peralta Delgado, Edmilson F de Oliveira-Filho, Mathieu Domenech de Cellès, Carlos Franco-Muñoz, María Paquita García Mendoza, Miladi Gatty Nogueira, Rosa-Margarita Gélvez-Ramírez, Manuel Gonzalez Gonzalez, Eduardo Gotuzzo, Stephanie Kramer-Schadt, Suvi Kuivanen, Katherine Laiton-Donato, Anyela Lozano-Parra, Edward Málaga-Trillo, Dora Valencia Manos Alva, Dorothée Missé, Andres Moreira-Soto, Thiago Moreno Souza, Karen Mozo, Eduardo Martins Netto, Nadine Olk, Johanna Maribel Pachamora Diaz, Célia Pedroso Jorge, Ana Micsuco Pérez Astudillo, Marta Piche-Ovares, Stephane Priet, Bladimiro Rincón-Orozco, Juan José Romero-Zúñiga, Silvia Paola Salgado Cisneros, Andreas Stöcker, Juan Carlos Villalobos Ugalde, Luis Angel Villar Centeno, Moritz Wenzler-Meya, Juan Carlos Zevallos, Jan Felix Drexler*

Summary

Background Latin America has been experiencing an Oropouche virus (OROV) outbreak of unprecedented magnitude and spread since 2023–24 for unknown reasons. We aimed to identify risk predictors of and areas at risk for OROV transmission.

Methods In this multidisciplinary, laboratory-based, modelling study, we retrospectively tested anonymised serum samples collected between 2001 and 2022 for studies on virus epidemiology and medical diagnostics in Bolivia, Brazil, Colombia, Costa Rica, Ecuador, and Peru with nucleoprotein-based commercial ELISAs for OROV-specific IgG and IgM antibodies. Serum samples positive for IgG from different ecological regions and sampling years were tested against Guaroa virus and two OROV glycoprotein reassortants (Iquitos virus and Madre de Dios virus) via plaque reduction neutralisation testing (PRNT) to validate IgG ELISA specificity and support antigenic cartography. Three OROV strains were included in the neutralisation testing, a Cuban OROV isolate from the 2023–24 outbreak, a contemporary Peruvian OROV isolate taken from a patient in 2020, and a historical OROV isolate from Brazil. We analysed the serological data alongside age, sex, cohort, and geographical residence data for the serum samples; reported OROV incidence data; and vector occurrence data to explore OROV transmission in ecologically different regions of Latin America. We used the MaxEnt machine learning methodology to spatially analyse and predict OROV infection risk across Latin America, fitting one model with presence–absence serological data (seropositive results were recorded as presence and seronegative results were recorded as absence) and one model with presence-only, reported incidence data from 2024. We computed marginal dependency plots, variable contribution, and permutation metrics to analyse the impact of socioecological predictors and fitted a generalised linear mixed-effects model with logit link and binary error structure to analyse the potential effects of age, sex, or cohort type bias and interactions between age or sex and cohort type in our serological data. We conducted antigenic cartography and evolutionary characterisations of all available genomic sequences for all three OROV genome segments from the National Center for Biotechnology Information, including branch-specific selection pressure analysis and the construction of OROV phylogenetic trees.

Findings In total, 9420 serum samples were included in this study, representing 76 provinces in the six Latin American countries previously mentioned. The sex distribution across the combined cohorts was 48% female (4237 of 8910 samples with available data) and 52% male (4673 of 8910 samples) and the mean age was 29·5 years (range 0–95 years). The samples were collected from census-based cohorts, cohorts of healthy individuals, and cohorts of febrile patients receiving routine health care. The average OROV IgG antibody detection rate was 6·3% (95% CI 5·8–6·8), with substantial regional heterogeneity. The presence–absence, serology-based model predicted high-risk areas for OROV transmission in the Amazon River basin, around the coastal and southern areas of Brazil, and in parts of central America and the Caribbean islands, consistent with case data from the 2023–24 outbreak reported by the Pan American Health Organization. Areas with a high predicted risk of OROV transmission with the serology-based model showed a statistically significant positive correlation with state-level incidence rates per 100 000 people in 2024 (generalised linear model, p=0·0003). The area under the curve estimates were 0·79 (95% CI 0·78–0·80) for the serology-based model and 0·66 (95% CI 0·65–0·66) for the presence-only incidence-based model. Longitudinal diagnostic testing of serum samples from cohorts of febrile patients suggested constant circulation of OROV in endemic regions at varying intensity. Climate variables accounted for more than 60% of variable contribution in both the serology-based and incidence-based models. Antigenic cartography, evolutionary

Lancet Infect Dis 2025;
25: 1020–32

https://doi.org/10.1016/
S1473-3099(25)00110-0

See **Comment** page 955

For the Spanish translation of the abstract see **Online** for appendix 1

For the Portuguese translation of the abstract see **Online** for appendix 2

*Contributed equally

Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Institute of Virology, Berlin, Germany (C Fischer MSc, A Frühauf MSc, M H Anzolini Cassiano MSc, E F de Oliveira-Filho PhD, S Kuivanen PhD, A Moreira-Soto PhD DVM, N Olk MSc, M Piche-Ovares MSc, Prof J F Drexler MD)**; Unité des Virus Émergents, Marseille, France** (L Inchauste PhD, K Barthélémy MSc, S Priet PhD, Prof X de Lamballerie MD PhD)**; Laboratorio Referencial de Salud Pública de San Martín, Tarapoto, Peru** (H Arévalo Ramirez MSc)**; Programa Nacional de Sangre, Ministerio de Salud y Deportes, La Paz, Bolivia** (L Bautista Machicado MSc)**; National Institute of Infectious Diseases Evandro Chagas, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil** (Prof F A Bozza MD PhD)**; CHRC, NOVA Medical School, Universidade Nova de Lisboa, Lisbon, Portugal** (Prof F A Bozza)**; Laboratório de**

analyses, and in-vitro growth comparisons showed clear differentiation between OROV and its glycoprotein reassortants, but not between the three different OROV strains. PRNT titres of OROV-neutralising serum samples were strongly correlated between all three tested OROV isolates (r>0·83; p<0·0001) but were not correlated with the two glycoprotein reassortants.

Interpretation **Our data suggest that climatic factors are major drivers of OROV spread and were potentially exacerbated during 2024 by extreme weather events. OROV glycoprotein reassortants, but not individual OROV strains, probably have distinct antigenicity. Preparedness for OROV outbreaks requires enhanced diagnostics, surveillance, and vector control in current and future endemic areas, which could all be informed by the risk predictions presented in this Article.**

Funding **European Union.**

Introduction

Oropouche virus (OROV) is an arthropod-borne virus (ie, arbovirus) endemic in Latin America. OROV is probably maintained in sylvatic and urban transmission cycles, both involving biting midges (genus *Culicoides*) as the primary vector. Since 1955, growing numbers of human OROV infections have been reported.[1] Since late 2023, Latin America has been experiencing an OROV outbreak of unprecedented magnitude, characterised by thousands of reported cases and a wide geographical spread, including in densely populated areas outside of the Amazon River basin (where OROV had previously mostly been detected). Sporadic adult and fetal deaths and congenital malformations have been reported,[2–4] and, in August, 2024, WHO declared OROV a high regional public health risk.[5]

Research in context

Evidence before this study

We searched PubMed on Nov 29, 2024, to review the available articles on Oropouche virus (OROV) published after October, 2023, when the 2023–24 OROV outbreak began. Use of the search strings "Oropouche virus" and "Oropouche" resulted in 90 entries, all of which were reviewed. The majority (60 [67%] of 90) of the reviewed articles were comments on the 2023–24 OROV outbreak or reviews of existing evidence. Original research providing new insights on OROV mostly focused on molecular detection of acute OROV infections in endemic settings and returning travellers, genomic characterisation of current OROV lineages, and analyses of symptoms and disease severity. In publications reporting original data, the 2023–24 outbreak was associated with a reassortant OROV lineage first detected in Brazil in 2015. OROV infections were sporadically linked to fetal malformations and abortions and adult deaths. Studies from several non-American countries reported OROV infections in returning travellers. Many factors were discussed as potential drivers of the 2023–24 OROV outbreak, but substantial uncertainty prevails. Ecological modelling, which was available only for Brazil, showed high OROV infection risk in the Amazon River basin and on the Brazilian coast.

Added value of this study

While other studies on the 2023–24 OROV outbreak have typically focused on analysing country-level geographical spread, individual socioecological predictors of OROV infection, or viral genomes, this is a multidisciplinary study combining in-vitro, serological, molecular, and genomic methods and spatial modelling with both serological data and reported incidence across Latin America. Our analyses rely on an unprecedented serological OROV dataset of more than 9000 samples covering six countries and multiple ecozones. We show that OROV is a common arboviral infection in the South American Amazon. OROV and OROV reassortants, but not individual OROV strains, might represent distinct serotypes. A strain from the 2023–24 OROV outbreak (IRCCS-SCDC_1/2024) did not stand out from either a historical or another contemporary strain in terms of its replication capacity or antigenicity. We analyse socioecological predictors of OROV transmission and provide spatial risk predictions for OROV transmission across Latin America. The risk estimates identified in this study are probably associated with the ecological niche of the main vector of OROV and cover areas that reported cases during the 2023–24 outbreak beyond the Amazon River basin (which is known to be a hot spot for OROV infections). Our findings provide substantial knowledge to help guide OROV containment measures and surveillance strategies.

Implications of all the available evidence

Our evidence shows that OROV infections are highly underestimated in Latin America. As climate predictors were the most important predictors in our OROV transmission risk models, OROV infection risk will probably evolve dynamically in the coming decades, with the potential for large-scale future outbreaks. Diagnostic testing strategies, clinical practices, vaccination research pipelines, and surveillance strategies should therefore prioritise OROV to mitigate this potential.

Pesquisa em Infectologia (A Stöcker MD), **Hospital Universitário Professor Edgard Santos** (Prof C Brites MD PhD, E Martins Netto MD PhD, C Pedroso Jorge PhD); **Universidade Federal de Bahia, Salvador, Brazil; Division of Infectious Diseases, Department of Internal Medicine, University of Texas Medical Branch, Galveston, TX, USA** (Prof M M Cabada MD); **Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru** (Prof M M Cabada, Prof E Gotuzzo MD, K Mozo MD MSc); **Centro Nacional de Salud Pública, Instituto Nacional de Salud, Peru** (C A Cabezas Sánchez MSc, M P García Mendoza MSc); **Caja Costarricense de Seguro Social, San José, Costa Rica** (A Cervantes Rodríguez MSc, J C Villalobos Ugalde MSc); **Dirección Regional de Salud de San Martín, Moyobamba, Peru** (R de los Milagros Peralta Delgado BSc, J M Pachamora Diaz NSc); **Infectious Disease Epidemiology Group, Max Planck Institute for Infection Biology, Berlin, Germany** (M Domenech de Cellès PhD); **Genomics of Emerging Microorganisms Group, Dirección de Investigación en Salud Pública, Instituto Nacional de Salud, Bogotá, Colombia** (C Franco-Muñoz MSc, K Laiton-Donato PhD); **Laboratorio de Referencia Regional de Salud Pública–Gerencia Regional de Salud, Loreto, Peru** (M Gatty Nogueira MSc); **Centro de Atención y Diagnóstico de Enfermedades Infecciosas, Fundación INFOVIDA, Red AEDES, Bucaramanga, Colombia** (R-M Gélvez-Ramírez PhD, L A Villar Centeno MSc); **Universidad ECOTEC, Samborondón, Ecuador** (M Gonzalez Gonzalez MSc); **Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany** (Prof S Kramer-Schadt PhD, M Wenzler-Meya MSc); **Institute of Ecology, Technische Universitaet Berlin, Berlin, Germany** (Prof S Kramer-Schadt); **Grupo Epidemiología Clínica**

(A Lozano-Parra PhD), **Department of Basic Sciences** (B Rincón-Orozco PhD), **School of Medicine, Universidad Industrial de Santander UIS, Red AEDES, Bucaramanga, Colombia; Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru** (E Málaga-Trillo PhD); **Laboratorio Referencial, Dirección Regional de Salud de Lambayeque, Chiclayo, Peru** (D V Manos Alva MSc); **MIVEGEC, IRD, University of Montpellier, CNRS, Montpellier, France** (D Missé PhD); **Fundação Oswaldo Cruz-Fiocruz, Centro de Desenvolvimento Tecnológico em Saúde, Instituto Nacional de Ciência e Tecnologia de Gestão da Inovação em Doenças Negligenciadas, Rio de Janeiro, Brasil** (T Moreno Souza PhD); **Laboratorio de Referencia Regional de Salud Pública–Dirección Regional de Salud Tumbes, Tumbes, Peru** (A M Pérez Astudillo MSc); **Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica** (J J Romero-Zúñiga PhD); **National Institute of Public Health Research, Guayaquil, Ecuador** (S P Salgado Cisneros MSc); **Health Science Faculty, Universidad Espíritu Santo, Guayaquil, Ecuador** (J C Zevallos MD); **German Centre for Infection Research, associated partner site Charité, Berlin, Germany** (Prof J F Drexler)

Correspondence to:
Prof Jan Felix Drexler, Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Institute of Virology, Berlin 10117, Germany
felix.drexler@charite.de

See **Online** for appendix 3

The potential drivers underlying the 2023–24 OROV outbreak that have been considered include, among others, a detection bias due to increased OROV testing;[6] a more virulent phenotype of the OROV outbreak strain coupled with antigenic differences that facilitate immune escape and reinfection;[7] a different vector (as has been reported in Cuba: *Culex quinquefasciatus* in addition to *Culicoides paraensis*);[1,8] land cover changes, such as deforestation;[9] and higher vector abundance and spread due to extreme weather events, such as El Niño, in Latin America.[10] Substantial uncertainty regarding the causes of the 2023–24 OROV outbreak persists, partly due to the absence of comprehensive data beyond reported incidence, including serological survey data.

We conducted a multidisciplinary study including serological testing, in-vitro comparisons of different OROV strains, phylogenetic analyses, and mathematical modelling to predict OROV transmission risks across Latin America and to identify socioecological predictors of OROV transmission.

Methods

Study design

We performed a multidisciplinary, laboratory-based, modelling study. We retrospectively tested anonymised serum samples already available from studies on virus epidemiology and medical diagnostics (appendix 3 p 2) for OROV-specific antibodies and analysed these serological data (alongside age, sex, cohort, and geographical residence data; reported OROV incidence and vector occurrence data; and all available OROV genomic data) to explore OROV transmission in ecologically different regions of Latin America (covering Bolivia, Brazil, Colombia, Costa Rica, Ecuador, and Peru). Coverage was dependent on access to samples. The only inclusion criteria for the serum samples were available information on the individual's area of residence and unique sample identifiers. Samples with missing age, sex, or collection year data were not excluded from analysis.

This study was approved under ethical permit EA2/031/22 from Charité–Universitätsmedizin Berlin. Sampling and sample testing was approved by the ethics committee of the Universidad Peruana Cayetano Heredia (permits R-087–06–24 and R-167–22–17), the Brazilian national ethics committee (Comissão Nacional de Ética em Pesquisa; permits 1.408.499, 2.744.551, and 2.869.566), the ethics committee of the Industrial University of Santander (Comité de Ética en Investigación Científica de la Universidad Industrial de Santander; permit 202124001448301), the Colombian ethics committee for biomedical research (Comité de Ética en Investigación Biomédica del CDI; permit 202124001473531), the human research ethics committee of the Espiritu Santo University (Comité de Ética de Investigación en Seres Humanos de la Universidad de Especialidades Espíritu Santo; permit 2022–002A), the Peruvian bioethics committee (Comité lnstitucional de Bioética; permits 6528 and 6532), the ethics committee of the National Health Institute of Colombia (Comité de Ética y Metodologías de la Investigación del Instituto Nacional de Salud; permit CEMIN 20–2022), the ethics committee of the Dr Mario Ortíz Suárez Hospital, Santa Cruz de la Sierra, Bolivia (number FWA00026426), and the National Health Research Council of the Costa Rican Health Ministry (CONIS-457-2021).

Laboratory testing

OROV-reactive IgG and IgM class antibodies were tested with nucleoprotein-based commercial ELISAs specific for IgG and IgM (Euroimmun, Luebeck, Germany). As defined by the manufacturer, ELISA ratios of more than 1·1 were considered positive. Although no human orthobunyavirus closely related to OROV is known in Latin America,[1] serum samples positive for IgG from different ecological regions and sampling years and representing the full range of ELISA reactivity were tested via plaque reduction neutralisation testing (PRNT) to validate IgG ELISA specificity and support antigenic cartography (appendix 3 pp 2–3). PRNT is the serological gold standard for arboviruses.[11] Serum samples were tested in two-fold dilutions between 1:20 and 1:5120 for their neutralisation of approximately 50 plaque-forming units of Guaroa virus (GROV), an American human-pathogenic orthobunyavirus from a different serogroup to OROV to confirm ELISA specificity, and 50 plaque-forming units of Iquitos virus (IQTV) and Madre de Dios virus (MDDV) to compare neutralisation of different human-pathogenic OROV glycoprotein reassortants. Three OROV lineages were included in the neutralisation testing, a Cuban OROV isolate from the 2023–24 outbreak, a contemporary Peruvian OROV isolate taken from a patient in 2020, and a historical OROV isolate from Brazil (appendix 3 p 4). PRNT endpoint titres were calculated in R (version 4.3.2) with a generalised linear model.

Spatial modelling

Each serum sample was assigned GPS coordinates at the smallest geographical resolution available on the basis of individuals' residence information (appendix 3 p 2). Reported 2024 state-level OROV incidence data up until Sept 15 were extracted from the Pan American Health Organization epidemiological alert for all Latin American countries except Brazil, for which Ministry of Health data were used (appendix 3 pp 3, 5–6, 12). 14 spatial explanatory predictors were included after variable selection, including minimisation of multicollinearity (appendix 3 pp 7–9, 12). The Global Biodiversity Information Facility database was used on July 22, 2024, to download the available data on locations of reported occurrence of vectors associated with OROV transmission (with no country restriction).

For the **Global Biodiversity Information Facility** see https://www.gbif.org/

We used the MaxEnt machine learning methodology to analyse and predict OROV infection risk across Latin America, fitting two types of models: one with our presence–absence serological data (with seropositive results recorded as presence and seronegative results recorded as absence); and one with presence-only, reported incidence data from 2024 (appendix 3 p 2–3).[12] MaxEnt was chosen because it has previously been shown to robustly model OROV geographical transmission risks in Brazil in comparison with other spatial modelling and machine learning approaches,[13] and because it allowed us to use both presence-only and presence–absence data.[13,14] To analyse the impact of socioecological predictors, marginal dependency plots, variable contribution, and permutation metrics were computed (appendix 3 p 3). The 10th-percentile training presence logistical threshold was chosen as a cutoff for high-risk versus low-risk estimates (appendix 3 p 3). This threshold, as calculated by default settings in MaxEnt, is a logistic prediction value below which the lowest 10% of training occurrence points fall.

Mixed-effects modelling

To analyse the potential effects of age, sex, or cohort type bias and the interaction between age or sex and cohort type in the serological data, we fitted a generalised linear mixed-effects model with logit link function and binary error structure. Cohort identity, which already contained country information, was used as the random effect on the intercept (appendix 3 p 3).

Genomic analysis

All available OROV genomic sequences for all three genome segments were retrieved from the National Center for Biotechnology Information on Aug 23, 2024. The search query was “Orthobunyavirus oropouche ense” (the name of the viral species to which OROV and its glycoprotein reassortants belong). Retrieved sequences were filtered for at least 95% completeness of coding regions. Translated sequences were aligned with MAFFT (version 7). Phylogenetic trees were constructed with MrBayes (version 3.1) and a WAG amino acid substitution model. Branch-specific selection pressure analyses were performed locally with the Hyphy open-source software package and the aBSREL test for episodic diversification. Underlying phylogenies were generated with RAxML with a GTR + G + I nucleotide substitution model, and varying numbers of strains across gene segments according to availability (170 strains across the S segment, 404 strains across the M segment, and 493 strains across the L segment).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

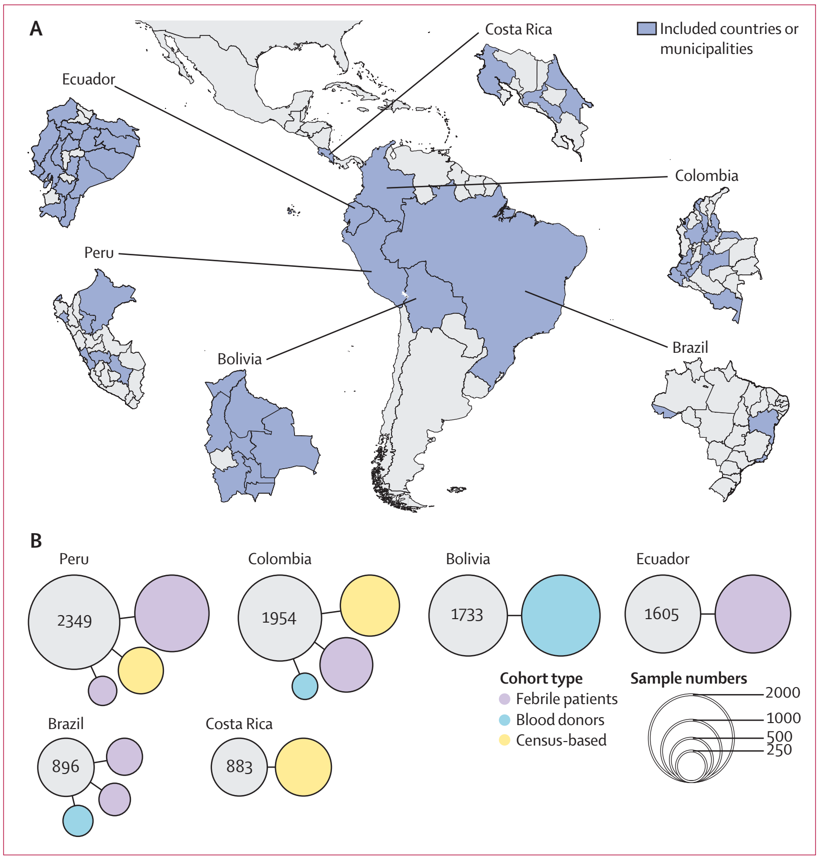


Figure 1: **Characteristics of the 9420 serum samples tested for Oropouche virus-specific IgG class antibodies**
(A) Spatial distribution of the samples. (B) Size and type of included cohorts.

Results

In total, 9420 serum samples collected between 2001 and 2022 were included in this study, representing 76 provinces in six Latin American countries (figure 1A; appendix 3 p 13). The sample set comprised 2171 samples from healthy individuals collected in Brazil, Colombia, and Bolivia; 2441 samples from individuals in census-based cohorts collected in Colombia, Costa Rica, and Peru for studies on COVID-19 and co-infections; and 4808 samples from febrile patients seeking routine health care collected in Brazil, Colombia, Ecuador, and Peru (figure 1B; table). The sex distribution across the combined cohorts was 48% female (4237 of 8910 samples with available data) and 52% male (4673 of 8910 samples). The mean age across the combined cohorts (N=8703 with available age data) was 29·5 years (range 0–95 years, 10–90 percentiles 8–55 years; appendix 3 p 13).

The average OROV IgG antibody detection rate was 6·3% (95% CI 5·8–6·8; figure 2A). In 43 (57%) of 76 provinces included in this study, serum samples tested positive for OROV-specific IgG. The median province-stratified detection rate of OROV-specific IgG antibodies varied significantly between countries (from 1% in Costa Rica to 5% in Ecuador; χ^2 test, p<0·0001) and within distinct ecological regions exemplified by

	Country	Samples	Cohort type	Geographical resolution*	Age	Sex	
						Female	Male
BRA_Acr	Brazil	254	Healthy	City level	40·8 (38·6–42·9; n=252)	57/254 (22%)	197/254 (78%)
BRA_Bah	Brazil	363	Febrile	City level	38·2 (36·9–39·5; n=358)	127/363 (35%)	236/363 (65%)
BRA_Rio	Brazil	279	Febrile	City level	..	..	..
COL_1	Colombia	986	Census-based	City level	39·4 (38·1–40·7; n=943)	354/943 (38%)	589/943 (62%)
COL_2	Colombia	184	Healthy	State level†	43·6 (41·8–45·4; n=184)	118/184 (64%)	66/184 (35%)
COL_3	Colombia	784	Febrile	District level‡	22·3 (21·4–23·1; n=784)	445/784 (57%)	339/784 (43%)
CR_CCS	Costa Rica	883	Census-based	District level	36·1 (34·5–37·6; n=620)	372/811 (46%)	439/811 (54%)
ECU	Ecuador	1605	Febrile	City level	19·5 (18·7–20·2; n=1605)	803/1605 (50%)	802/1605 (50%)
PER_1	Peru	226	Febrile	District level	17·9 (16·0–19·9; n=187)	92/187 (49%)	95/187 (51%)
PER_2	Peru	1551	Febrile	State level	26·6 (25·6–27·6; n=1477)	706/1475 (48%)	769/1475 (52%)
PER_3	Peru	572	Census-based	Province level§	35·7 (34·0–37·4; n=572)	217/572 (38%)	355/572 (62%)
BOL	Bolivia	1733	Healthy	Province level	31·2 (30·7–31·7; n=1721)	946/1024 (92%)	786/1024 (8%)
All cohorts	..	9420	..	..	29·5 (29·1–29·9; n=8703)	4237/8910 (48%)	4673/8910 (52%)

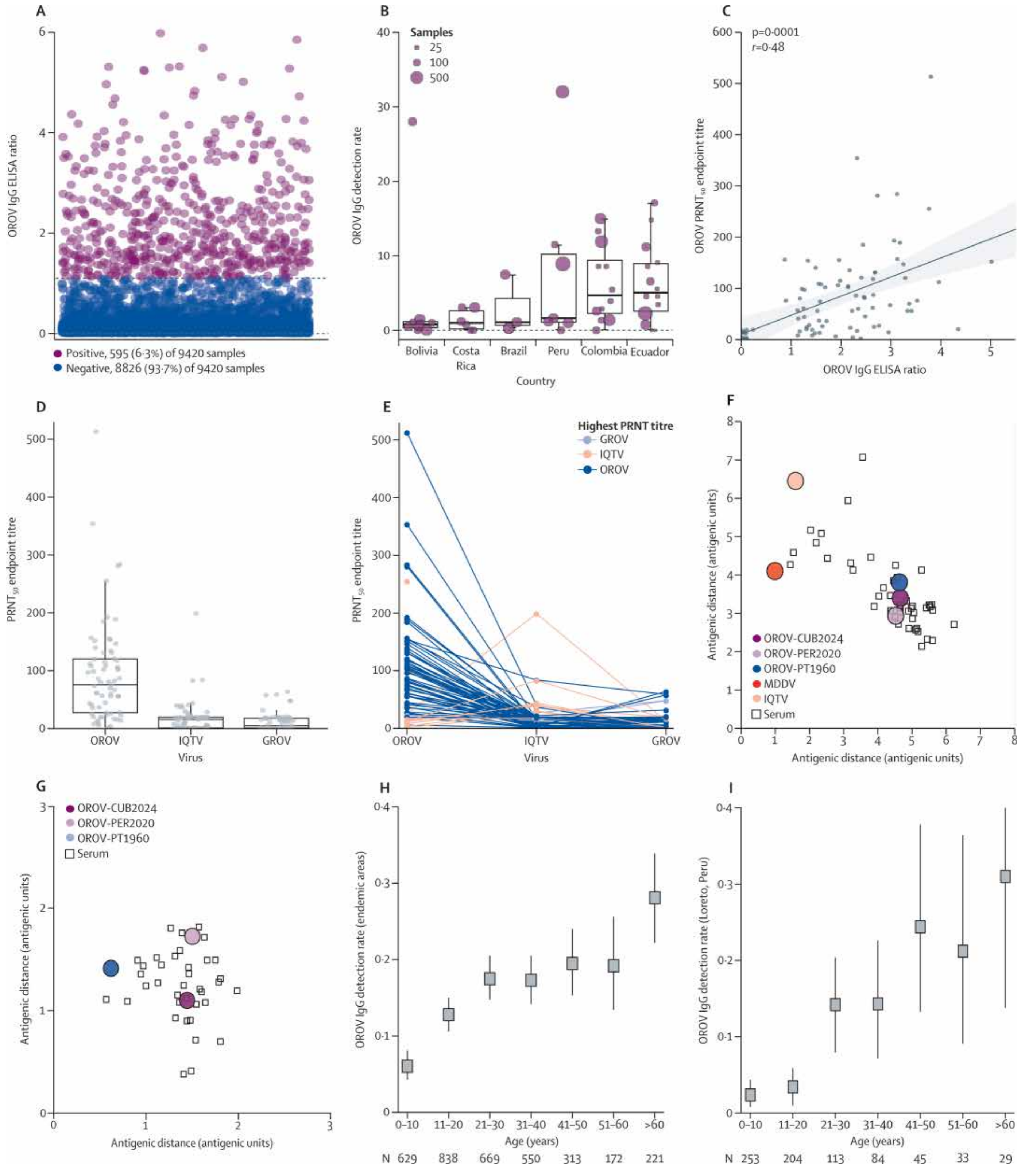
Data are n, mean (95% CI), or n/N (%). *Whenever lower-level administrative information was not unique, we used state-level polygons. If lower-level administrative information was missing, higher-level information was used. †First administrative level. ‡Third administrative level. §Second administrative level.

***Table:* Characteristics of study cohorts**

differences between country provinces (eg, below 1% in high-altitude Andean departments and above 10% in Amazonian settings; p<0·0001; figure 2B). To validate the robustness of the ELISA-based testing, a subset of 71 IgG ELISA-positive samples collected in Brazil, Colombia, Costa Rica, and Peru over a timespan of over 20 years (2002–22) was tested via PRNT. IgG ELISA ratios were significantly correlated with OROV-specific PRNT$_{50}$ titres (Pearson's correlation r=0·48; p<0·0001) but not with GROV-specific (p=0·21) or IQTV-specific (p=0·077) titres (figure 2C). Among the 71 samples tested via PRNT, the median PRNT$_{50}$ titres were 94 (95% CI 75–116) for OROV, 41 (95% CI 13–91) for IQTV, and 12 (95% CI 8–15) for GROV. Comparative endpoint titre testing for OROV, IQTV, and GROV revealed that 59 (83%) of the 71 samples (collected from Brazil, Colombia, Costa Rica, and Peru) had highest neutralisation titres for OROV (figure 2D–E), whereas ten (14%) of the 71 IgG ELISA-reactive samples collected from Brazil, Colombia, and Peru, but not Costa Rica, showed highest neutralisation of IQTV.[1,15] Among PRNT-confirmed samples, the IQTV detection rate was similar between Brazil (four [27%] of 15; 95% CI 7–48), Colombia (three [17%] of 18, 0–33), and Peru (three [10%] of 30, 0–23). Only two (3%) of 71 tested samples showed highest neutralisation titres for GROV, indicating a high specificity of the applied ELISA for OROV and its M segment reassortants, such as IQTV, which share S segments encoding the N antigen employed by the ELISA. This high specificity combined with the continuous IgG ELISA ratios among the 71 tested samples might indicate that we slightly underestimated the true OROV seropositivity in all regions (figure 2A). The inclusion of samples from febrile individuals in the IgG testing might affect the OROV-specific IgG detection rate due to overall increased exposure to arboviruses associated with areas of residence and socioeconomic factors.[16] Therefore, to validate our serological testing, we explored whether cohort type (ie, febrile, census-based, or healthy), sex, and age affected the IgG detection rate. Although statistically significant interactions between some variables occurred (appendix 3 pp 10, 14), no statistically significant effect of a febrile cohort type was observed on the OROV-specific IgG ELISA outcome (generalised linear mixed-effects model, p=0·31; appendix 3 p 10). Across all samples, the OROV-specific IgG detection rate was significantly correlated with age, in line with endemic OROV transmission and long-lived IgG responses following exposure (Pearson's correlation, p<0·0001, r=0·07; figure 2H, I).

***Figure 2:* Detection of OROV-specific IgG antibodies and comparative neutralisation of OROV strains and reassortants**

(A) OROV IgG ELISA ratios. (B) OROV IgG ELISA detection rate by province and country. (C) Correlation between IgG ELISA ratios and OROV neutralisation. (D) PRNT titres of IgG ELISA-positive samples for OROV, IQTV, and GROV. (E) PRNT titre comparison of IgG ELISA-positive samples for OROV, IQTV, and GROV. (F) Antigenic cartography of IgG ELISA-positive samples for three OROV strains and glycoprotein reassortants (MDDV and IQTV). (G) Antigenic cartography of IgG ELISA-positive samples for three OROV strains not including IQTV and MDDV. Both axes in panels F and G show antigenic distance with each grid square representing one antigenic unit (ie, a two-fold change in neutralisation titre). (H) Age-stratified OROV-specific IgG detection among all cohorts with at least a 5% detection rate. (I) Age-stratified OROV-specific IgG detection in Loreto, Peru, the studied province with the largest number of samples and at least a 5% detection rate. GROV=Guaroa virus. IQTV=Iquitos virus. MDDV=Madre de Dios virus. OROV=Oropouche virus. OROV-CUB2024=OROV isolated from serum of an Italian tourist returning from Cuba in 2024. OROV-PER2020=OROV isolated from febrile patient in Peru in 2020. OROV-PT1960=prototypic OROV derived from BeAn19991 isolated in 1960 with unclear passage history. The prototypic virus strain is a defined reference strain. PRNT=plaque reduction neutralisation testing.


A
OROV IgG ELISA ratio
6
4
2
0
Positive, 595 (6·3%) of 9420 samples
Negative, 8826 (93·7%) of 9420 samples
B
Samples
25
100
500
OROV IgG detection rate
40
30
20
10
0
Bolivia
Costa Rica
Brazil
Peru
Colombia
Ecuador
Country
C
p=0·0001
r=0·48
OROV PRNT$_{50}$ endpoint titre
600
500
400
300
200
100
0
0
1
2
3
4
5
OROV IgG ELISA ratio
D
PRNT$_{50}$ endpoint titre
500
400
300
200
100
0
OROV
IQTV
GROV
Virus
E
Highest PRNT titre
GROV
IQTV
OROV
PRNT$_{50}$ endpoint titre
500
400
300
200
100
0
OROV
IQTV
GROV
Virus
F
Antigenic distance (antigenic units)
8
7
6
5
4
3
2
1
0
OROV-CUB2024
OROV-PER2020
OROV-PT1960
MDDV
IQTV
Serum
0
1
2
3
4
5
6
7
8
Antigenic distance (antigenic units)
G
OROV-CUB2024
OROV-PER2020
OROV-PT1960
Serum
Antigenic distance (antigenic units)
3
2
1
0
0
1
2
3
Antigenic distance (antigenic units)
H
OROV IgG detection rate (endemic areas)
0·4
0·3
0·2
0·1
0
0–10
11–20
21–30
31–40
41–50
51–60
>60
Age (years)
N 629
838
669
550
313
172
221
I
OROV IgG detection rate (Loreto, Peru)
0·4
0·3
0·2
0·1
0
0–10
11–20
21–30
31–40
41–50
51–60
>60
Age (years)
N 253
204
113
84
45
33
29

The 2023–24 OROV outbreak strains were hypothesised to evade pre-existing immunity and to be more replication competent than other OROV strains. However, genomic distances for this strain are small for all three segments, particularly on the amino-acid level (appendix 3 p 14).[7,17]

To explore neutralisation of OROV and its reassortants, 46 serum samples collected between 2002 and 2020 in Brazil, Colombia, Costa Rica, and Peru were tested by PRNT$_{50}$ against a historical OROV isolate from Brazil in 1960 (BeAn19991), a genetically diverse contemporary

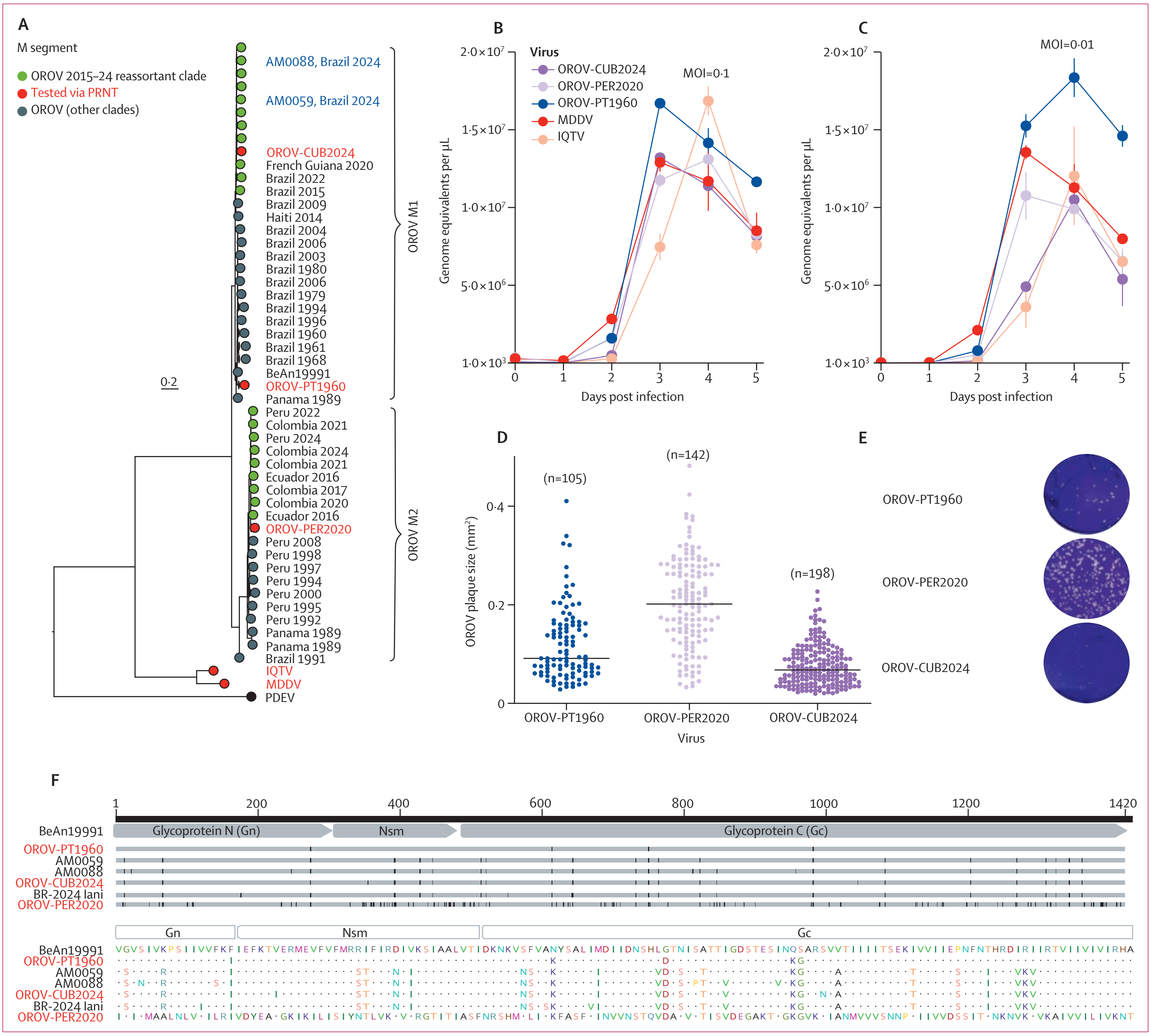


Figure 3: Phylogenetic and in-vitro comparison of Oropouche viruses

(A) Phylogeny of Oropouche virus M segments. For clarity of presentation, viruses are identified by their country and year of isolation with the exception of selected OROV strains, prototypic strain PT1960, IQTV, MDDV, and the outgroup PDEV. Major glycoprotein lineages M1 and M2 are shown next to the brackets. (B) Growth kinetics of selected OROV strains and reassortants in Vero E6 cells infected with an MOI of 0·1. (C) Growth kinetics of selected OROV strains and reassortants in Vero E6 cells infected with an MOI of 0·01. (D) Average plaque size of different OROV strains. (E) Plaque morphology of OROV strains. (F) Amino acid exchanges in selected Oropouche viruses in glycoproteins and the non-structural Nsm protein encoded by the M segment, in comparison with the BeAn19991 reference sequence. AM0059=OROV isolate AM0059/Brazil/2024 (GenBank accession PP992528). AM0088=OROV isolate AM0088/Brazil/2024 (GenBank accession PP992527). BeAn19991=a historical OROV isolate from Brazil in 1960. BR-2024 Iani=OROV isolate OROV_AC_04.24_2024 (GenBank accession PQ168299). IQTV=Iquitos virus. MDDV=Madre de Dios virus. MOI=multiplicity of infection. OROV=Oropouche virus. OROV-CUB2024=OROV isolated from serum of an Italian tourist returning from Cuba in 2024. OROV-PER2020=OROV isolated from febrile patient in Peru in 2020. OROV-PT1960=prototypic OROV derived from BeAn19991 isolated in 1960 with unclear passage history. PDEV=Perdões virus. PRNT=plaque reduction neutralisation testing.

OROV isolate from Peru, a 2023–24 outbreak OROV isolate from Cuba, covering most antigenic and phylogenetic OROV diversity (particularly regarding the M segment), and the two human-pathogenic reassortants IQTV and MDDV (figure 3A, appendix 3 p 15). Compared with the historical BeAn19991 strain, the Cuban 2023–24 outbreak isolate and the contemporary Peruvian isolate are 98·2% and 95·7% conserved (averaged across all viral proteins), respectively, at the amino-acid level. In contrast, IQTV and MDDV are only 85·0% and 82·7% conserved, respectively, compared with OROV BeAn19991. The Cuban isolate shares 99·7% identity on the amino-acid level with Brazilian isolates from 2024, differing in 11 amino acids of which only two differences occur in the major glycoprotein Gc, which is the most important target of neutralising antibodies in orthobunyaviruses (figure 3F; appendix 3 p 16).[18] PRNT$_{50}$ titres were strongly correlated between all three tested OROV isolates (Pearson's correlation, p<0·0001, *r*>0·83). Neutralisation titres against IQTV and MDDV were strongly correlated (Pearson's correlation, p<0·0001, *r*>0·99) but neither was correlated with OROV PRNT$_{50}$ (Pearson's correlation, p>0·05, *r*<0·22) or PRNT$_{90}$ titres (appendix 3 p 11). Similarly, in antigenic cartography, the three OROV isolates clustered closely together within less than one antigenic unit, whereas IQTV and MDDV were clearly separated from OROV by approximately three antigenic units. The antigenic distance between IQTV and MDDV was roughly two antigenic units (figure 2F–G; appendix 3 p 15). Neutralisation patterns were comparable among samples collected before 2015, when the reassortant OROV 2015–24 clade was first detected, and samples collected after 2015 (appendix 3 p 15).

Similarly to the results for neutralisation by human sera, the 2023–24 outbreak-associated OROV isolate did not stand out from other OROV isolates in terms of its growth kinetics and plaque size. At two different multiplicities of infection (a ratio that compares the number of inoculated virus to the number of target cells), the 2024 outbreak-associated strain grew identically or slower than the prototype and contemporary strains (figure 3B, C). Median plaque sizes were significantly smaller for the Cuban 2024 outbreak-associated strain than for the contemporary Peruvian strain (*t* test, p<0·0001; figure 3D, E). No evidence for diversifying evolution segregating the outbreak clade from other OROV clades was found in analyses of the OROV genomic segments (appendix 3 p 17).

To investigate potential variation in OROV infection rates between different seasons and years,[19] we tested serum samples from four Peruvian regions collected from patients with acute febrile disease in at least 2 different years with real-time RT-PCR and IgM ELISA for OROV (appendix 3 p 2). In total, 110 (7·7%) of 1422 samples tested positive: 25 (1·8%) of 1422 tested positive via PCR and 85 (6·0%) of 1422 tested positive via IgM ELISA. OROV incidence varied significantly among regions, ranging from 1·7% to 17·7%, and between different years, ranging from 6% (56 of 965, 95% CI 4–7) to 22% (16 of 73, 12–32; χ^2 test, p<0·0001) (figure 4 A–E; appendix 3 p 10), confirming regional and yearly differences in OROV transmission activity in line with previous investigations from the Peruvian Amazon.[17,20] Together, molecular and serological testing suggest that OROV is sustained constantly with seasonal human OROV infections in endemic regions such as the Amazon River basin. Substantial incidence in multiple years, evidence for OROV transmission in areas that have never reported infections, higher than 10% IgG detection rates in Amazonian settings, and the stringent increase of detection rates with age are in stark contrast to a few hundred reported cases in several Latin American countries before and during 2024, indicating stark under-reporting of OROV infections.[7]

To understand spatial risk patterns of OROV infection across Latin America, we fitted two models with presence–absence serological data and 2024 presence-only incidence data based on acute cases (figure 5A–C, appendix 3 p 18). The area under the curve estimates were 0·79

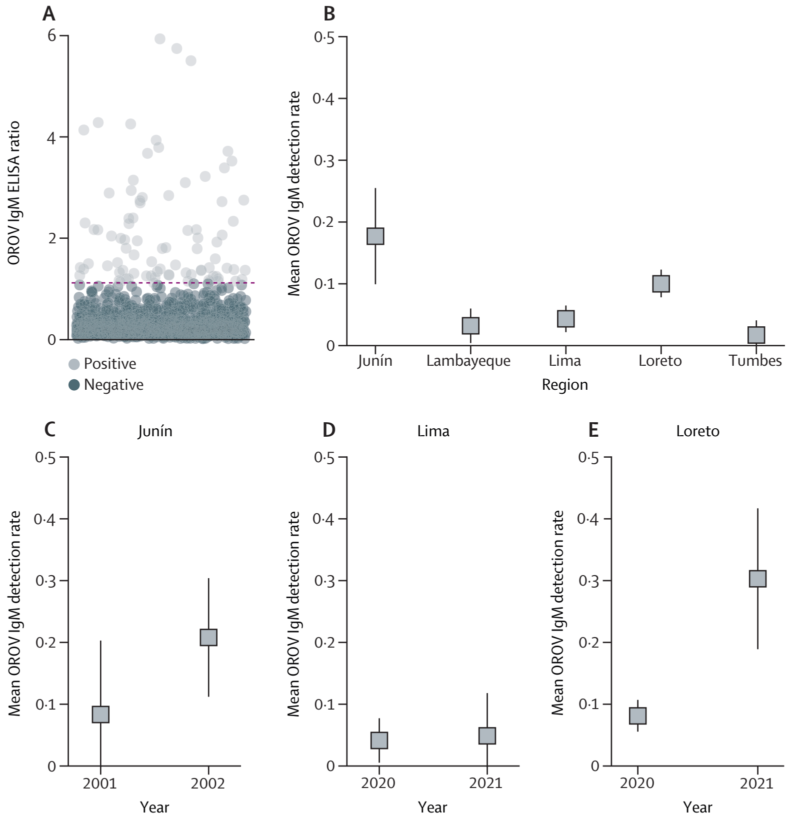
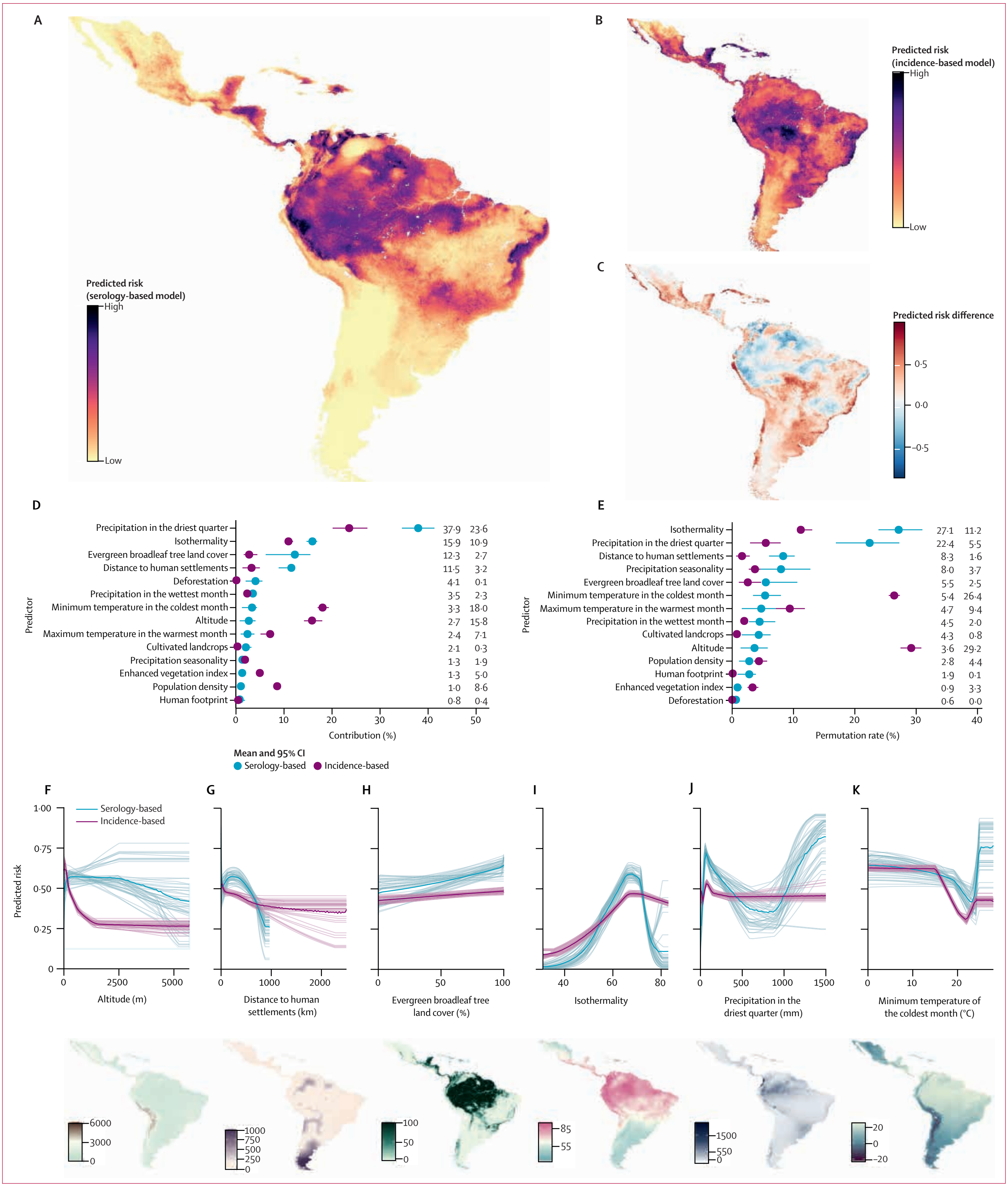


Figure 4: **Detection of acute OROV infections in Peru**
(A) OROV-specific IgM ELISA ratios. The dotted line indicates the 1·1 threshold above which results are considered positive according to the manufacturer's instructions. (B) Average OROV detection rate. Retrospective detection of OROV infections in 2001 and 2002 in Junín (C), in 2020 and 2021 in Lima (D), and in 2020 and 2021 in Loreto (E). Bars indicate 95% CIs (B–E). OROV=Oropouche virus.


A
Predicted risk
(serology-based model)
High
Low
B
Predicted risk
(incidence-based model)
High
Low
C
Predicted risk difference
0·5
0·0
–0·5
D
Predictor
Precipitation in the driest quarter 37·9 23·6
Isothermality 15·9 10·9
Evergreen broadleaf tree land cover 12·3 2·7
Distance to human settlements 11·5 3·2
Deforestation 4·1 0·1
Precipitation in the wettest month 3·5 2·3
Minimum temperature in the coldest month 3·3 18·0
Altitude 2·7 15·8
Maximum temperature in the warmest month 2·4 7·1
Cultivated landcrops 2·1 0·3
Precipitation seasonality 1·3 1·9
Enhanced vegetation index 1·3 5·0
Population density 1·0 8·6
Human footprint 0·8 0·4
0
10
20
30
40
50
Contribution (%)
E
Predictor
Isothermality 27·1 11·2
Precipitation in the driest quarter 22·4 5·5
Distance to human settlements 8·3 1·6
Precipitation seasonality 8·0 3·7
Evergreen broadleaf tree land cover 5·5 2·5
Minimum temperature in the coldest month 5·4 26·4
Maximum temperature in the warmest month 4·7 9·4
Precipitation in the wettest month 4·5 2·0
Cultivated landcrops 4·3 0·8
Altitude 3·6 29·2
Population density 2·8 4·4
Human footprint 1·9 0·1
Enhanced vegetation index 0·9 3·3
Deforestation 0·6 0·0
0
10
20
30
40
Permutation rate (%)
Mean and 95% CI
● Serology-based ● Incidence-based
F
G
H
I
J
K
Serology-based
Incidence-based
Predicted risk
1·00
0·75
0·50
0·25
0
0
2500
5000
Altitude (m)
0
1000
2000
Distance to human settlements (km)
0
50
100
Evergreen broadleaf tree land cover (%)
40
60
80
Isothermality
0
500
1000
1500
Precipitation in the driest quarter (mm)
0
10
20
Minimum temperature of the coldest month (°C)
6000
3000
0
1000
750
500
250
0
100
50
0
85
55
1500
550
0
20
0
–20

(95% CI 0·78–0·80) for the serology-based model and 0·66 (0·65–0·66) for the incidence-based model. When running the trained models to predict OROV transmission risk across Latin America, both showed a high risk in the Amazon River basin, around the coastal and southern areas of Brazil, and in parts of central America and the Caribbean islands (figure 5A). The serology-based model predicted high OROV transmission risk for areas where no serological data had been collected but cases had been reported in 2024 (appendix 3 p 18) and individual risk values in the serology-based and incidence-based models also indicated a statistically significant moderate positive correlation (Spearman's rank correlation, *r*=0·54, p<0·0001). Although predicted risk values in the serology-based model showed a statistically significant positive correlation with state-level incidence rates per 100 000 people in 2024 (generalised linear model, p=0·0003), some states deviate from model predictions (appendix 3 p 19), which could characterise changes in risk patterns during the 2023–24 outbreak or reporting biases. In comparison to the serology-based model, the incidence-based model predicted a higher general risk across Latin America, which might indicate risk overestimation potentially linked to the use of presence-only incidence data biased by the concentration of medical care and laboratory diagnostics in urban settings (figure 5A–C). In particular, the incidence-based model predicted a higher risk of OROV infection around the southeastern part of the Amazon River basin, the coastlines of Brazil and Chile, and in parts of central America and the Caribbean islands, and lower risk in areas towards the northern parts of South America, partly contradicting reported cases during the 2023–24 outbreak (figure 5A–C).

In both the serology-based and incidence-based models, climate variables, including isothermality (an indicator of the constancy of temperature across seasons), and precipitation variables were the most important predictors, characterised by high permutation and variable contribution estimates (figure 5D–K; appendix 3 p 19). In total, climate variables accounted for more than 60% of variable contribution in the models. In both models, distance to human settlements (a proxy for anthropogenic land cover changes such as deforestation) and evergreen broadleaf tree land cover were the only predictors related to human infrastructure that showed at least 10% variable contribution. Deforestation contributed to less than 4·5% of variable contribution in both models (figure 5D–K; appendix 3 p 11).[9] Marginal dependency plots showed a negative association between altitude and distance to human settlements and predicted risk, whereas for evergreen broadleaf tree land cover, precipitation, and isothermality, a positive association was observed (figure 5F–K). These findings might be similar to the conditions commonly found in tropical settings, such as minimal seasonality, abundant rainfall, and extensive evergreen vegetation, which could be associated with increased transmission risk in these climates and ecozones. When comparing the available occurrence data for vectors to our risk predictions, only *C paraensis* had a mean risk prediction above the high-risk cutoff of 0·30 (95% CI 0·29–0·31) in the serology-based model, despite having fewer reported occurrences (n=95) than the other vectors. In contrast, the average predicted risk for GPS points with reported vector occurrences was significantly higher for all other vectors in the incidence-based model (Wilcoxon signed-rank test, p<0·0001; appendix 3 p 20). This finding could be explained by a recent change in vector range or, more probably, reporting biases affecting the model.

Discussion

In this study, we investigated OROV transmission patterns across Latin America and show that climatic factors probably contribute substantially to the spread of OROV. Our findings, including an unprecedented serological survey, provide evidence that OROV infections in Latin America have been consistently under-reported, which is supported by retrospective detections of OROV during dengue fever outbreaks.[6,21]

Comparative neutralisation testing did not confirm escape from antibody-mediated immunity as a major driver for OROV transmission, which had been indicated in a previous study.[7] Although high protein similarities between the tested OROV lineages might support cross-neutralisation among OROV lineages, further studies are needed to explore differences between studies, including the time since primary OROV infection (potentially allowing for repeated infection with OROV) and potential artifacts of high-passage isolates, highlighting the relevance of using contemporary low-passage strains for comparative testing. Beyond immune escape, genetic adaptations could also affect OROV replication.[22,23] At least two different OROV lineages are currently co-endemic in the Amazon rainforest.[24] Surveillance in co-endemic regions and in-vivo infection studies could verify

***Figure 5:* Seasonal variation of OROV incidence and machine learning-based spatial risk prediction for central and South America**

(A) Risk prediction with the model fitted with presence–absence serological data. (B) Risk prediction with the model fitted with presence-only incidence data. In both cases, risk predictions are averages of five model runs (appendix 3 p 18). (C) Predicted risk difference between the incidence-based and serology-based models. Blue characterises a decreased risk in the incidence-based model compared with the serology-based model. Red indicates an increase in risk. (D) Relative variable contribution of single variables in the two models. (E) Permutation estimates of single variables in the two models. (F–K) Marginal dependency plots for all predictors with more than 10% variable contribution in at least one model. The thick lines characterise the mean response of 50 replicate MaxEnt runs, whereas the thin lines characterise one of 50 individual model iterations. The fluctuation of predicted risk at extreme endpoints is probably attributable to the scarcity of predictor data at such values, especially in the serology-based model, which is based on true presence–absence data with an overall smaller N in comparison with the incidence-based model, for which pseudo-absences were generated (appendix 3 pp 2–3).

in-vitro findings and provide important data on whether OROV lineages and reassortants differ in attack rate, effective reproduction number, and regional occurrence patterns compared with other lineages.[1] In this study, antigenic distances between OROV and OROV glycoprotein reassortants have been found to be comparable to those discriminating between dengue virus serotypes,[25] suggesting that MDDV and IQTV probably represent at least one antigenically distinct group from OROV.

Beyond potential viral adaptation, our spatial models emphasise the importance of climatic predictors linked to rainfall and temperature variation. With climatic variables being the most important predictors explaining OROV transmission risk in our models, the influence of extreme weather events, including El Niño, probably played a key role in the 2023–24 OROV outbreak.[17,26] Changing weather conditions could favour increased OROV transmission by increasing the number of vector populations, favouring transovarial transmission, or enhancing viral replication in ectothermic vectors (as has been seen with dengue virus, which spread to high-altitude regions that had previously acted as transmission barriers).[27] In regions with a high estimated risk for OROV transmission where OROV cases have not yet been reported, increased surveillance is crucial to fully comprehend and respond to the current and future OROV outbreaks.

In addition to climatic factors, changes in land use and land cover can influence vector abundance. For example, logging and fires in the Brazilian Amazon have been linked to increased malarial vector occurrence and incidence.[28] Although their influence was less pronounced than that of climatic variables in our analyses on OROV, factors such as proximity to human settlements and evergreen broadleaf tree land cover showed some contribution in our models, in line with the correlation found between reported OROV cases in Brazil in 2024 and proximity to agricultural plantations such as palm oil plantations, which are commonly found close to rain forests.[29] The minimal contribution of deforestation in our models could hypothetically be attributed to OROV cases predominantly occurring in areas that have already (or recently) been deforested and underscores the importance of obtaining granular, up-to-date deforestation data. Although we explored a number of land cover variables, long-term cohort studies and vector studies exploring the abundance of OROV vectors along a gradient of recent deforestation and land use change are necessary.

Our serology-based spatial model shows high discriminative ability, highlighting the value of employing cross-sectional serological datasets in the prevention of infectious outbreaks and epidemic responses, despite their complexity and cost.[13,30] In contrast, incidence data can be biased by geographically heterogeneous testing, especially in the early phases of outbreaks when testing infrastructure in remote areas and awareness of emerging pathogens are scarce.[31] Ideally, future modelling of incidence rates during outbreaks should incorporate negative testing data to reduce the effect of methodological choices, such as the creation of pseudo-absence points, on modelling outputs.

This study has several limitations. Our serological results are limited by the absence of PRNT confirmation for all IgG ELISA-positive samples and the uncertain durability of OROV-specific IgG antibody responses. However, IQTV detection rates were similar in the Amazon regions of Brazil, Colombia, and Peru. The ecology of IQTV, including its spread by *Culicoides* vectors, and its reported geographical occurrence are probably very similar to those of OROV, minimising the potential impact of IQTV detection on our predictions.[1] Detection of OROV-specific IgG was correlated with age, suggesting long-lived antibody responses and supporting the endemicity of OROV. Data scarcity and quality limited our geospatial predictions and modelling, potentially introducing selection, information, and reporting biases. For example, age data could not be included in spatial models and continent-wide climate data were not available for 2024. Although diverse, unrepresentative cohorts and varying reporting periods for predictors were used, our subanalyses showed that cohort type had no significant effect on OROV occurrence. When possible, we conducted sensitivity analyses and adjusted variable selection across models for different time periods.

Overall, our findings highlight that OROV is a majorly neglected arbovirus and that climate variables and, to an unclear extent, anthropogenic land cover changes are main drivers of its transmission. Our results indicate that OROV outbreaks are likely to occur with strong variation in case numbers and geographical spread depending on environmental and weather conditions. Regions without known OROV circulation are at risk of becoming endemic for OROV, as was observed during the 2023–24 outbreak. Our findings emphasise the need to prioritise Oropouche fever in regional diagnostic testing and surveillance frameworks, which can be informed by our risk predictions; conduct experimental and epidemiological vector studies; adapt vector control to include midges; develop vaccines; and increase awareness among potentially endangered communities, clinicians, and public health stakeholders.

Contributors

AF, CF, and JFD conceptualised this study; AF, CF, LI, MW-M, and SP were responsible for data curation; AF, CF, and MHAC did the formal analysis; JFD was responsible for funding acquisition; AF, CF, JFD, and LI were responsible for the investigation; AF, CF, JFD, LI, SK-S, and SP were responsible for the methodology; AF, AM-S, CF, and JFD were responsible for project administration; ACR, AF, AL-P, AM-S, AS, BR-O, CACS, CB, CF-M, CF, CPJ, DM, DVMA, EG, EMN, EM-T, EFdO-F, FAB, HAR, JCVU, JCZ, JFD, JJRZ, JMPD, KB, KL-D, KM, LAVC, LBM, LI, MMC, MDdC, MGG, MGN, MHAC, MPGM, MP-O, MW-M, NO, RdlMPD, R-MG-R, SK, SK-S, SP, SPSC, TMS, and XdL were responsible for resources; AF, AM-S, CF, MHAC, and

MW-M were responsible for software; JFD, MDdC, and SK-S supervised this study; MDdC, MW-M, and SK-S verified the data; AF, CF, EFdO-F, and MHAC prepared the figures; AF, CF, and JFD wrote the original draft manuscript; all authors reviewed and edited the draft manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

Binary ELISA results with information on age and state of residence only (for data protection), risk predictions, and R scripts can be accessed via GitHub. Virus accession numbers are provided in appendix 3 (p 4).

For the **GitHub repository** see https://github.com/drexler-virus-epidemiology/OROV

Acknowledgments

We would like to thank our colleagues who provided insight and expertise that greatly assisted our research: Alejandro Alfaro-Alarcón (Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Institute of Virology, Berlin, Germany and Departamento de Patología, Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica); Stephan Hartmann (Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Institute of Virology, Berlin, Germany); Arne Kühne (Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Institute of Virology, Berlin, Germany); Dominik Lehmann (Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Institute of Virology, Berlin, Germany); Valeria Penner (Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Institute of Virology, Berlin, Germany); Liena de Regla Ponce Rey (Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Institute of Virology, Berlin, Germany and Faculty of Biology, University of Havana. Plaza de la Revolución, Havana, Cuba); Nazaret Ruiz-Villalobos, Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Institute of Virology, Berlin, Germany and Programa de Investigación en Enfermedades Tropicales, Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica). This study was supported by the EU via the project Zoonosis Emergence across Degraded and Restored Forest Ecosystems (ZOE, project number 101135094), which was awarded to JFD. Additional funding originated from the National Institutes of Health (grant U19AI181984); the Global Centers for Health and Pandemic Prevention via the German Academic Exchange Services (DAAD) project 57592642 (GLACIER) to JFD; the Berlin Institute of Health at Charité via a BIH-MD-Stipend to AF; a DAAD stipend to MP-O; the German Ministry of Education and Research within the framework of the project Arbofusion (project number 01DN19017A) to JFD, MMC, and EG; and the German Federal Ministry of Economic Cooperation and Development via the Deutsche Gesellschaft für Internationale Zusammenarbeit (project number 81262528) to JFD, EM-T, MPGM, and CACS. Work in Peru by MMC received funding from the Global Infectious Diseases Research Network at the Center for Tropical Diseases, University of Texas Medical Branch at Galveston, TX, USA. Work in Colombia by BR-O received funding from the Universidad Industrial de Santander and the Ministerio de Ciencias de Colombia (MinCiencias project numbers BPIN 2020000100126, 112189684486, and CT 629–2021). The Oropouche virus strain IRCCS-SCDC_1/2024 was provided by Concetta Castilletti, Department of Infectious Tropical Diseases and Microbiology, IRCCS Sacro Cuore Don Calabria Hospital, Negrar di Valpolicella, Verona, Italy. The Iquitos virus and Madre de Dios virus were provided by the World Reference Center for Emerging Viruses and Arboviruses through the University of Texas Medical Branch at Galveston, TX, USA.

Editorial note: The Lancet Group takes a neutral position with respect to territorial claims in published maps and institutional affiliations.

References

1 Wesselmann KM, Postigo-Hidalgo I, Pezzi L, et al. Emergence of Oropouche fever in Latin America: a narrative review. *Lancet Infect Dis* 2024; **24:** e439–52.

2 Samara A, Coutinho CM, Veal P, et al. Potential vertical transmission of Oropouche virus during the current outbreak. *Lancet Infect Dis* 2024; **24:** e668–69.

3 das Neves Martins FE, Chiang JO, Nunes BTD, et al. Newborns with microcephaly in Brazil and potential vertical transmission of Oropouche virus: a case series. *Lancet Infect Dis* 2025; **25:** 155–65.

4 Bandeira AC, Pereira FM, Leal A, et al. Fatal Oropouche virus infections in nonendemic region, Brazil, 2024. *Emerg Infect Dis* 2024; **30:** 2370–74.

5 WHO. Oropouche virus disease—region of the Americas. World Health Organization, 2024. https://www.who.int/emergencies/disease-outbreak-news/item/2024-DON530 (accessed Oct 11, 2024).

6 Gaillet M, Pichard C, Restrepo J, et al. Outbreak of Oropouche virus in French Guiana. *Emerg Infect Dis* 2021; **27:** 2711–14.

7 Scachetti GC, Forato J, Claro IM, et al. Re-emergence of Oropouche virus between 2023 and 2024 in Brazil: an observational epidemiological study. *Lancet Infect Dis* 2024.

8 Benitez AJ, Alvarez M, Perez L, et al. Oropouche fever, Cuba, May 2024. *Emerg Infect Dis* 2024; **30:** 2155–59.

9 Moreira HM, Sgorlon G, Queiroz JAS, et al. Outbreak of Oropouche virus in frontier regions in western Amazon. *Microbiol Spectr* 2024; **12:** e0162923.

10 Pan American Health Organization. Dengue cases. Health Information Platform for the Americas, Pan American Health Organization, 2024. https://www3.paho.org/data/index.php/en/mnu-topics/indicadores-dengue-en.html (accessed March 28, 2025).

11 Fischer C, Jo WK, Haage V, Moreira-Soto A, de Oliveira Filho EF, Drexler JF. Challenges towards serologic diagnostics of emerging arboviruses. *Clin Microbiol Infect* 2021; **27:** 1221–29.

12 Phillips SJ, Anderson RP, Schapire RE. Maximum entropy modeling of species geographic distributions. *Ecol Modell* 2006; **190:** 231–59.

13 Tegally H, Dellicour S, Poongavanan J, et al. Dynamics and ecology of a multi-stage expansion of Oropouche virus in Brazil. *medRxiv* 2024; published online Oct 30. https://doi.org/10.1101/2024.10.29.24316328 (preprint).

14 Kramer-Schadt S, Niedballa J, Pilgrim JD, et al. The importance of correcting for sampling bias in MaxEnt species distribution models. *Divers Distrib* 2013; **19:** 1366–79.

15 Baer K, Arora I, Kimbro J, et al. Iquitos virus in traveler returning to the United States from Ecuador. *Emerg Infect Dis* 2024; **30:** 2447–51.

16 Braga C, Martelli CMT, Souza WV, et al. Seroprevalence of dengue, chikungunya and Zika at the epicenter of the congenital microcephaly epidemic in northeast Brazil: a population-based survey. *PLoS Negl Trop Dis* 2023; **17:** e0011270.

17 Naveca FG, Almeida TAP, Souza V, et al. Human outbreaks of a novel reassortant Oropouche virus in the Brazilian Amazon region. *Nat Med* 2024; **30:** 3509–21.

18 Hellert J, Aebischer A, Wernike K, et al. Orthobunyavirus spike architecture and recognition by neutralizing antibodies. *Nat Commun* 2019; **10:** 879.

19 Martins-Filho PR, Soares-Neto RF, de Oliveira-Júnior JM, Alves Dos Santos C. The underdiagnosed threat of oropouche fever amidst dengue epidemics in Brazil. *Lancet Reg Health Am* 2024; **32:** 100718.

20 Watts DM, Russell KL, Wooster MT, et al. Etiologies of acute undifferentiated febrile illnesses in and near Iquitos from 1993 to 1999 in the Amazon River basin of Peru. *Am J Trop Med Hyg* 2022; **107:** 1114–28.

21 Cardoso BF, Serra OP, Heinen LB, et al. Detection of Oropouche virus segment S in patients and in *Culex quinquefasciatus* in the state of Mato Grosso, Brazil. *Mem Inst Oswaldo Cruz* 2015; **110:** 745–54.

22 Ciota AT, Kramer LD. Insights into arbovirus evolution and adaptation from experimental studies. *Viruses* 2010; **2:** 2594–617.

23 Rodriguez-Morales AJ, Drexler JF. Re-emergence of Oropouche virus in Brazil and Latin America. *Lancet Infect Dis* 2025; **25:** 137–39.

24 Usuga J, Limonta D, Perez-Restrepo LS, et al. Co-circulation of 2 Oropouche virus lineages, Amazon Basin, Colombia, 2024. *Emerg Infect Dis* 2024; **30:** 2375–80.

25 Katzelnick LC, Fonville JM, Gromowski GD, et al. Dengue viruses cluster antigenically but not as discrete serotypes. *Science* 2015; **349:** 1338–43.

26 World Meteorological Organization. El Niño and climate change impacts slam Latin America and Caribbean in 2023. World Meteorological Organization, May 24, 2024.

27 Barcellos C, Matos V, Lana RM, Lowe R. Climate change, thermal anomalies, and the recent progression of dengue in Brazil. *Sci Rep* 2024; **14:** 5948.

28 MacDonald AJ, Mordecai EA. Amazon deforestation drives malaria transmission, and malaria burden reduces forest clearing. *Proc Natl Acad Sci USA* 2019; **116:** 22212–18.

29 Gräf T, Delatorre E, do Nascimento Ferreira C, et al. Expansion of Oropouche virus in non-endemic Brazilian regions: analysis of genomic characterisation and ecological drivers. *Lancet Infect Dis* 2024; published online Nov 15. https://doi.org/10.1016/S1473-3099(24)00687-X.

30 Romero-Alvarez D, Escobar LE, Auguste AJ, Del Valle SY, Manore CA. Transmission risk of Oropouche fever across the Americas. *Infect Dis Poverty* 2023; **12:** 47.

31 Worsley-Tonks KEL, Bender JB, Deem SL, et al. Strengthening global health security by improving disease surveillance in remote rural areas of low-income and middle-income countries. *Lancet Glob Health* 2022; **10:** e579–84.