nature portfolio

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# Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

## Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed | |
|---|---|---|
| ☐ | ☒ | The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☒ | ☐ | The statistical test(s) used AND whether they are one- or two-sided<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☐ | ☒ | A description of all covariates tested |
| ☒ | ☐ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☒ | ☐ | For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*Give P values as exact values whenever suitable.* |
| ☐ | ☒ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ | Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

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|---|---|
| Data collection | Data acquisition on the Luminex MagPix instrument was performed using the software xPONENT v.4.3 update 2 |
| Data analysis | Custom code was developed in R (version 4.3.3.). Bayesian finite mixture models were executed from R on the Stan platform (version 2.35) using the cmdstanr package (version 0.7.1) as the R interface to Stan. Mathematical models of antibody cross-reactivity and virus transmission were implemented in Python (v. 3.13.1) using the PyMC module (v. 5.20.0). Code is available under an MIT license from https://github.com/ymanvictor/arbo_multiplex |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

## Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The raw pseudo-anonymised individual-level data generated in this study are protected and are not available due to data privacy laws. The processed source data and data-derivatives sufficient to reproduce the analyses are included within the supplementary material (source_data.zip) or available from https://

doi.org/10.5281/zenodo.18704896. To allow the individual-level to be securely queried, we have developed a publicly available HTML widget that allows readers to explore all pairwise combinations of flavivirus and alphavirus antibody responses (https://ymanvictor.github.io/arbo_antibody_browser).

## Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

| | |
|---|---|
| Reporting on sex and gender | This work is based on a secondary analysis of samples previously collected in other studies.<br><br>All constituent cross-sectional surveys collected data on self-reported sex. Counts of female and male participants in each survey are provided below under "Population Characteristics" and within Table 1 of the manuscript.<br><br>Sex was not included explicitly in the main analysis of the manuscript because the primary objectives were to estimate the population-average seroprevalence, antibody levels, and force of infection, irrespective of sex. At each site, the sex distribution of the archived cross-sectional samples reflects that of the underlying population, therefore the resulting marginal (population-level) estimates are appropriate for this objective. Accordingly, inference from the GMM-based serostatus classification and the virus transmission and cross-reactivity model is presented for the overall population, with uncertainty quantified using posterior credible intervals.<br><br>Age-specific antibody levels stratified by sex and study site are presented in Supplementary Fig. 5. An accompanying HTML widget allows readers to explore all pairwise combinations of flavivirus and alphavirus antibody responses at each site and within each sex stratum (https://ymanvictor.github.io/arbo_antibody_browser). |
| Reporting on race, ethnicity, or other socially relevant groupings | Socially constructed categorization variables were not used within the manuscript. |
| Population characteristics | Cross-sectional surveys<br>Peru (Iquitos) 2018 (n=880; female: 59.0%; age-range: 0-102)<br>French Guiana 2015 (n=409; female: 29.8%; age-range: 18-62)<br>French Guiana 2019 (n=378; female: 27.0%; age-range: 18-70)<br>Senegal (Dielmo) 2016 (n=311; 51.8; age-range: 5-75)<br>Senegal (Dielmo) 2018 (n = 207; female: 51.2; age-range: 6-94)<br>Senegal (Ndiop) 2016 (n=385; female: 63.1; age-range: 0-82)<br>Senegal (Ndiop) 2018 (n=316; female: 63.1%; age-range: 6-77)<br>New Caledonia 2021-22 (n=600; female: 59%; age-range: 18-64) |
| Recruitment | No participants were recruited and no new samples were collected as part of this study. This study involved secondary use of samples previously collected in other studies.<br>In Peru, 880 samples were obtained from an all-age cross-sectional population survey of arboviruses and malaria conducted in 2018 in three peri-urban communities in Iquitos district, Department of Loreto, in the Peruvian Amazon.<br>In Senegal, 1,219 samples were obtained from 4 population-level cross-sectional malaria prevalence surveys conducted in 2016 and 2018 in villages Dielmo and Ndiop.<br>In French Guiana, 787 samples were obtained from two multicentric cross-sectional surveys conducted in Maroni area along the French Guiana-Suriname border in 2015 (n = 409) and in 2019 (n = 378) to study the prevalence of malaria in adult goldminers.<br>In New Caledonia 600 samples were obtained from adults 18-64 years of age participating in the 2021-2022 Adult Health Barometer, a cross-sectional descriptive survey. |
| Ethics oversight | No new samples were collected as part of this study. This study involved secondary use of samples previously collected in other studies. For all studies, the ethical approval and the informed consent covered this additional analysis.<br><br>The surveys in French Guiana were approved by the Commissie voor Mensgebonden Wetenschappelijk Onderzoek (CMWO) in Suriname (Opinion Number VG10-14 & DVG-738) and by the Institut national de la santé et de la recherche médicale (INSERM) Ethics Evaluation Committee in France (No. 14-187 of 09.12.2014).<br>The Peruvian samples were collected from participants in the study Circles of Research on Arboviruses and Malaria (SIDISI 101645/2017), following approval by the Institutional Ethics Committee of Universidad Peruana Cayetano Heredia (UPCH).<br>The Senegalese samples were collected as part of the ongoing Dielmo and Ndiop project which was examined and approved by the Senegalese National Health Research Ethics Committee (CNERS Sénégal). Approval to measure antibodies to arboviruses in these samples was granted by CNERS Sénégal (N 00000007 MSAS/CNERS/Sec). The study in New Caledonia was approved by the "Comité de Protection des Personnes Ouest IV" (21.05.16.92458-avis_50_21_2 and avis_50-21_2MS1) and by the Consultative Ethics Committee of New Caledonia (Avis 2021-03 002). The study was recorded on Clinicaltrials.gov (ID: NCT05218304). The negative French samples were obtained from the COVID-Oise study which was registered with ClinicalTrials.gov (NCT04644159) and received ethical approval from the Comité de Protection des Personnes Nord Ouest IV. The negative Peruvian samples were collected from healthy individuals from Lima as part of the Molecular and Sero-epidemiology of relapsing Plasmodium vivax in Peru (SIDISI 100873/2017) study, which was approved by the Institutional Ethics Committee of UPCH. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

# Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

# Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

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|---|---|
| Sample size | Total sample size, n = 4084<br><br>Cross-sectional surveys<br>Peru (Iquitos) (2018: n=880)<br>French Guiana (2015: n=409; 2019: n=378)<br>Senegal (Dielmo) (2016: n=311; 2018: n = 207)<br>Senegal (Ndiop) (2016: n=385; 2018: n=316)<br>New Caledonia (2021-22: n=600)<br><br>Validation cohorts<br>France (Unexposed: n= 249)<br>Peru (Unexposed: n = 89)<br>Peru (Suspected exposed: n = 102)<br>Senegal (Suspected exposed: n = 158)<br><br>This observational study was based on archived samples and data. The sample size was determined by the number of available samples from each site and survey and a prospective sample size/power calculation was therefore not applicable.<br>Nonetheless, the Bayesian framework—reporting uncertainty via posterior credible intervals—together with the large overall sample spanning a broad age range and multiple sites yields stable estimates for the study's primary objectives. |
| Data exclusions | No data was excluded from the analysis |
| Replication | The same technical controls were included in all experiments to monitor plate to plate, day to day, batch to batch, and operator related variation, and to normalize data to adjust for technical effects. Normalization procedures are described in the Methods. Across runs performed on different days, plates, and operators, technical control performance and resulting normalized values were consistent (overall control CV < 20%). Most samples were analysed in a single replciate. A subset of 155 samples from Senegal were analysed at two separate ocassions by two distinct researchers (JR and LG) using two different Luminex instruments (Luminex MagPix vs Luminex Intelliflex) with highly concordant results (spearman's rho >0.935 across all antigens). |
| Randomization | This study is observational in nature. There was no intervention and participants were not allocated into experimental groups |
| Blinding | This study is observational in nature and no intervention or grouping was applied. Thus, no formal blinding was required. Nevertheless, investigators were blinded to sample exposure status throughout the laboratority analysis of arboviral antibody responses. |

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☐ | ☒ Antibodies |
| ☒ | ☐ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology and archaeology |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |
| ☒ | ☐ Plants |

## Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☒ | ☐ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

## Antibodies

Antibodies used: Phycoerytrin (PE) conjugated F(ab')2 fragment Donkey anti-Human IgG, Jackson ImmunoResearch: JIR709116098)

Validation: Only commercially available secondary antibodies were used. As per the manufacturer's website: "Based on immunoelectrophoresis and/or ELISA, the antibody reacts with the Fc portion of human IgG heavy chain but not with the Fab portion of human IgG. No antibody was detected against human IgM or IgA, or against non-immunoglobulin serum proteins. The antibody has been tested by ELISA and/or solid-phase adsorbed to ensure minimal cross-reaction with bovine, horse and mouse serum proteins, but it may cross-react with immunoglobulins from other species.". In all experiments the secondary antibody was diluted 1:120.

## Plants

Seed stocks: *Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*

Novel plant genotypes: *Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*

Authentication: *Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*