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G. Pakoa , P. Guyant , C. Inizan , M. White , J. Vanhomwegen ,
M. Dupont-Rouzeyrol

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Highlights

- About a third of Vanuatu's population has been exposed to dengue, varying by age.
- Immunity (IgG) was very low for Zika, and very rare or absent for chikungunya, yellow fever, West Nile and Japanese Encephalitis
- Anti-Ross River virus IgG antibodies in 8.6% of the population indicates undetected Ross River virus circulation in Vanuatu.
- Persistent malaria transmission, with seroprevalence varying by island and age.

Title

A multiplex serological survey highlights differences in population immunity to mosquito-borne diseases in Vanuatu, Pacific region, 2023.

Authors and affiliations

K. Huet^{1,*°}, W. Donald^{2,*}, A. Kalulu², L. Vigier², D. Hoinard³, L. Garcia⁴, AF. Griffon¹, O. O'Connor¹, C. Depuille⁵, T. B. Knox^{6,7}, G. Pakoa², P. Guyant^{6,8}, C. Inizan¹, M. White⁴, J. Vanhomwegen³, M. Dupont-Rouzeyrol^{1°}

* co-first author

° corresponding author

1: Institut Pasteur of New Caledonia, URE Dengue and Arboviruses, Pasteur Network, 98800 Noumea, New Caledonia

2. Ministry of Health, Port-Vila, Vanuatu

3: Environment and Infectious Risks Research and Expertise Unit, Institut Pasteur, Université Paris Cité, 75015 Paris, France

4. Infectious Disease Epidemiology and Analytics G5 Unit, Institut Pasteur, Université Paris Cité, INSERM U1347, 75015 Paris, France

5. Pacific Community, Melanesia Regional Office, Port-Vila, Vanuatu;

6. World Health Organization, Vanuatu Country Liaison Office, Port-Vila, Vanuatu;

7. James Cook University (PacMOSSI consortium), Cairns 4870, Australia;

8. World Health Organization, Division of Pacific Technical Support, Suva, Fiji.

Abstract

Objectives: Mosquito-borne pathogens are major emerging diseases and a growing public health concern in the Pacific, with occurrences linked to global increases, climate change and human mobility. To support risk assessment of arboviruses and malaria in Vanuatu, a seroprevalence survey was conducted on Espiritu Santo Island, Efate Island, Malekula Island and Tanna Island between October 2022 and May 2023.

Methods: Serum samples from 1,120 healthy participants aged 6–84 were tested for IgG against multiple arboviruses and malaria antigens using a multiplex microsphere immunoassay.

Results: Standardized seroprevalence (IgG) reached 34% for dengue virus (DENV), 3.2% for Zika virus (ZIKV), 8.6% for Ross River virus (RRV), 4.4% for *Plasmodium falciparum*, and 36% for *Plasmodium vivax*. Significant differences were observed between islands for ZIKV and *P. vivax*, and between age groups for DENV, RRV, and *P. falciparum*. Seroprevalence was very rare or absent for West Nile virus, Japanese Encephalitis virus, Yellow fever virus and chikungunya virus.

Conclusions: These results suggest silent circulation of RRV, consistent with findings in Fiji and French Polynesia, and confirm ongoing malaria transmission. In a context of climate change and rapid spread of infectious diseases, our data suggest a significant risk for Vanuatu's population if pathogens were introduced. This risk should be further assessed according to the presence and distribution of competent vectors and on their potential for local transmission.

Keywords

Seroprevalence, Dengue, Ross River, Malaria, Pacific, Arboviruses, Vanuatu

Introduction

Vector-borne diseases transmitted by insects like mosquitoes and ticks threaten over 80% of the world's population. In particular, malaria and arboviruses represent the main mosquito-borne public health concerns worldwide [1]. Malaria is caused by *Plasmodium* parasites transmitted by *Anopheles* mosquitoes, with *Plasmodium falciparum* and *Plasmodium vivax* causing most cases [2]. Clinical presentations range from mild fever to life-threatening complications [3]. Two pediatric vaccines are currently available. Growing artemisinin-based resistance against standard treatments remains a major concern [4]. On the other hand, arboviruses from the *Orthoflavivirus* genus—such as dengue (DENV), Zika (ZIKV), yellow fever (YFV), Japanese encephalitis (JEV) or West Nile (WNV)—cause conditions ranging from mild joint pain to fatal hemorrhagic or encephalitis fevers and viruses from the *Alphavirus* genus, including Chikungunya (CHIKV) and Ross River (RRV), trigger severe rashes and persistent polyarthritis which can lead to death in the case of CHIKV infections [5]. These viruses are primarily transmitted by mosquitoes from the *Aedes* and *Culex* genera [5]. Furthermore, while vaccines exist for diseases like YFV, JEV, DENV and CHIKV, there are currently no specific antiviral treatments available.

The Pacific region is characterized by a vast area of open ocean with thousands of Islands scattered in between, which comprise the 22 Pacific Island Countries and Territories (PICTs) [6]. The geography, tropical climate and presence of competent vectors make the Pacific

region particularly vulnerable to epidemics of mosquito-borne diseases [6]. In the Pacific region, malaria is reported only in Papua New Guinea (PNG), the Solomon Islands and Vanuatu, with the highest estimated malaria mortality rates in the former two (36.3 and 16.9 per 100,000 people at risk for PNG and the Solomon Islands, respectively) and no deaths in Vanuatu since 2012 [7]. Epidemiological data indicate that DENV is the major arbovirus circulating in the Pacific region [8]. Until the year 2000, DENV circulation in the Pacific displayed a 5-years cyclical pattern of occurrence characterized by alternating transmission of each of the DENV serotypes (DENV-1 to -4). However, since the beginning of 2004, substantial changes in the epidemiology of arboviruses in the Pacific occurred with the co-circulation of different DENV-serotypes along with either ZIKV or CHIKV [8]. Furthermore, in the 2014-2020 period, 104 unique arboviral outbreaks occurred in the Pacific including 72 DENV outbreaks affecting 19 PICTs, 14 CHIKV outbreaks affecting 11 PICTs, and 18 ZIKV outbreaks affecting 14 PICTs [9]. In 2025, DENV remained a major public health issue in the Pacific with more than 20,000 confirmed cases, 3,900 hospitalizations and 21 deaths mainly due to DENV-1 and DENV-2 [10]. With the exception of Australia, where it has been in regular circulation for several years, RRV circulation has only been recorded in the Pacific in 1980, with about 500,000 cases notified in New Caledonia, Fiji, Wallis and Futuna, American Samoa and the Cook Islands. Finally, YFV circulation has never been documented in the Pacific to date, and JEV and WNV have only been found in PNG and Australia, but never in the islands further east of these two countries [11].

Vanuatu is an archipelago located between the Solomon Islands, New Caledonia and Fiji Islands. It comprises 83 Islands grouped into six provinces and is home to about 310,000 inhabitants, 99% of whom are Melanesian [12]. Eighty percent of the population live on 12

major Islands, and the two main cities are Port Vila on Efate Island and Luganville on Espiritu Santo Island. Vanuatu disease surveillance relies on syndromic surveillance through a designated network of sentinel sites across its six provinces. This network includes diseases such as influenza-like illness, dengue-like illness, acute fever and rash, acute diarrhea and others. Malaria and dengue are notifiable diseases and are reported to the Ministry of Health. Despite continuous efforts and digital modernization, disease surveillance in Vanuatu faces significant structural and geographical challenges. This includes transport of specimen from provincial to the national reference laboratory in Port Vila, limited laboratory infrastructure as confirmatory testing for some pathogens is restricted at the national reference laboratory and samples need to be sent abroad (like Australia or New Caledonia), exposition to natural disasters (cyclones, volcanic eruption, earthquakes) which can damage infrastructure or routine surveillance activities and, finally, under-reporting of cases. However, Vanuatu had accomplished substantial progress in malaria control, with cases decreasing from around 15,000 in 2003 to 322 in 2021, with no reported malaria deaths since 2012 [13]. In 2017, malaria elimination was declared in the province of Tafea which includes the Island of Tanna Island by the Vanuatu Ministry of Health after three years with no detection of local cases. However, nationwide confirmed cases surged from 312 in 2021 to 1,102 in 2022 and further increased to 2,261 cases in 2023. A drop was noted for 2024, with 1,576 confirmed cases reported [14]. Although WNV, YFV and JEV have never been detected in Vanuatu, DENV and ZIKV circulation have been reported, with DENV-1 causing the first DENV outbreak reported in 1972 and DENV-2 causing the most recent DENV outbreak reported in 2021, and the first ZIKV circulation in 2014 [15]. Contrary to other PICTs, CHIKV or RRV have not been detected in Vanuatu. Vaccines against *P. falciparum* malaria or arboviruses are not currently in use in public health programs in Vanuatu.

Serosurveys are epidemiological studies measuring the proportion of the population having antibodies against specific pathogens, indicating population exposure and a potential protective immunity. These studies provide essential data on the proportion of the population that has been exposed to diseases, thereby helping to estimate the true burden of the disease, which might be underreported through routine surveillance systems. Moreover, seroprevalence data can identify high-risk groups and geographic areas with higher transmission rates, informing targeted interventions [16]. In Vanuatu, the frequency and intensity of arboviruses outbreaks and the upsurge of malaria have highlighted the urgent need for comprehensive epidemiological studies to inform public health strategies and control measures. In this context, this study aimed at determining the seroprevalence of arboviruses and malaria in 2023 in Vanuatu. Our findings could be used to assess the level of epidemic risk based on past exposure and to update surveillance and laboratory systems. These results can also provide health security data to adapt prevention and public health intervention strategies to protect the population of Vanuatu.

Methods

Study population

This study is a non-comparative observational prospective cross-sectional descriptive survey on a representative random sample of the population. The study was carried out on Efate Island (Shefa province), Espiritu Santo Island (Sanma province), Malekula Island (Malampa province) and Tanna Island (Tafea province), which are the four most populated provinces of Vanuatu and represent 74.5% of Vanuatu's population. Additional criteria included available dengue surveillance data, ease of access for logistical purposes, the presence of a nearby

health facility, and the feasibility of mobilizing health workers and community health volunteers. For schools, the same criteria were applied and included also a partnership with the Health Promoting Schools Program. The study thus was implemented in the main towns of Efate, Espiritu Santo, Tanna and Malekula islands, namely Port Vila, Luganville, Isangel and Lakatoro along with other villages and rural parts connected to the main city by main roads. The sample size of 1,200 participants was determined to ensure a maximum absolute precision of $d = 0.04$ at a 95% confidence level ($Z = 1.96$), assuming a conservative seroprevalence of $P = 50\%$ ($n_{\text{theoretical}} = 600$). To account for potential cluster effects inherent to complex multi-site sampling, a design effect (DEFF) of 1.5 was applied. Furthermore, assuming a non-response rate of 50%, 1,800 individuals were invited to achieve the final targeted 1,200 included individuals. Representativeness is ensured through stratified quota sampling based on the 2020 Vanuatu Census data [12] proportional to age, and provincial population distributions across major urban and rural epidemiological hotspots (Efate, Espiritu Santo, Tanna, and Malekula). Five age groups were constituted: 6-18, 19-34, 35-44, 45-64, and 65 and over. Community mobilization and information activities were conducted before field data collection. Training sessions for community mobilization were organized for council area administrators, chiefs, community-based leaders, area secretaries, volunteer health workers, nurses, Health Promoting School Coordinators, and school directors at the selected sites. The training aimed to ensure that local stakeholders understood the study objectives and could effectively inform and mobilize the population. A communication package (in English, Bislama and French) was provided to support community awareness of the survey. Participants from 6- to 84-years-old were then informed and asked to participate voluntarily in the study in October 2022 (Espiritu Santo and Malekula islands), November 2022 and February 2023 (Efate island) and May 2023 (Tanna island). Children aged 6 to 18 were included

in schools and high schools. Adults were included either in villages through punctual inclusion campaigns or among non-febrile patients or relatives of patients referring to the hospitals and health facilities in the different locations. Inclusions occurred in 20 sites (including six schools): seven in Efate Island, five in Espiritu Santo Island, four in Malekula Island and four in Tanna Island. Exclusions criteria were as following: tourists and people present in Vanuatu for less than 6 months, persons suffering from a febrile syndrome, persons intellectually disabled or under guardianship, or missing information related to age or hometown. After informed consent was provided, a blood sample was collected, and a questionnaire for socio-demographic data was administered (age, sex, living village/city). Serum was retrieved by centrifugation and stored at -20°C until subsequent analysis.

Multiplex immunoassay for arboviruses and malaria

We used a multiplex microsphere immunoassay (MIA) to detect IgG antibodies against a panel of mosquito-borne pathogens. This 12-plex test, applied to heat-inactivated serum diluted 1:400, enables the simultaneous detection of IgG targeting the envelope domain III (EDIII) of DENV (4 serotypes), WNV, JEV, YFV, ZIKV, the soluble envelope glycoprotein 2 (E2) for CHIKV and RRV[17], and the merozoite surface protein 1 (MSP1) antigen for *P. falciparum* and *P. vivax*. Samples were analyzed using a Luminex MagPix or Labscan100 system for arboviruses and malaria, with antibody levels measured in units of Median Fluorescent Intensity (MFI). Arbovirus antigens were expressed as SNAP tagged fusion proteins to facilitate oriented covalent coupling as previously described [18]. Measured antibody levels to arbovirus antigens were normalized by dividing each MFI by the measured antibody response to the SNAP tag alone, resulting in units of Relative Fluorescence Intensity (RFI). Cut-offs for ZIKV, CHIKV and DENV were previously established using a multicentric study of well-characterized

arbovirus-seropositive serum collections [17,19] and were set at 2.7 with sensitivity of 94.1% (86.7 - 98.0%) and specificity of 92.6% (86.8 - 96.4%) for ZIKV; at 3.6 with sensitivity of 95.6% (87.6 - 99.1%) and specificity of 98.7% (95.3 - 99.8%) for CHIKV and 2.7 with sensitivity of 94.2% (91.0 - 96.5%) and specificity of 96.6% (91.5 - 99.1%) for DENV. A serum was considered IgG positive for DENV if at least one of the four DENV antigens (EDIII from DENV-1 to DENV-4) tested positive. For non-endemic *Orthoflaviviruses* (i.e., JEV, WNV and YFV), the RFI cut-off was set at 4.0 to maximize assay specificity. The cut-off for RRV was adjusted and set at 3.6 using 141 sera from previous collections characterized for RRV by both ELISA (enzyme-linked immunosorbent assay) and seroneutralization. Only double-positive and double-negative sera were retained to determine the cut-offs using a ROC curve (Supp Figure 1), setting sensitivity at 83.3% (95% CI [79.8-86.3]) and specificity at 95.3% (95% CI [92.4-97.2]). The *Plasmodium* cut-offs were determined using the mean plus three standard deviations of negative sera from samples from New Caledonia, where there is no malaria transmission detected.

Statistical analysis

To account for the complex survey design and ensure data representativeness, seroprevalence estimates were adjusted using post-stratification weighting based on the 2020 Vanuatu Census [12]. Sampling weights were computed for each stratum defined by province and age group as the ratio of the target population proportion to the observed sample proportion. Weights were subsequently normalized relative to the sum of the weights for each age group in each province ($n = 20$), maintaining an average weight of 1 without altering the variance or the point estimates. All reported national and age-specific seroprevalence rates were calculated using these normalized post-stratification weights to reflect the target population structure. Seroprevalence for each arbovirus and *Plasmodium* was calculated as the weighted

proportion of participants with detectable antibodies against the mosquito-borne pathogen tested. Analyses of the cohort were carried out for the five age classes used for weighting data, according to gender (self-declaration) and according to the four provinces sampled. We used the “Survey” package in R studio (R 4.2.1) software to calculate the weighted proportion of the population positive for each mosquito-borne pathogen studied. Proportions were compared using Pearson's Chi-squared statistical test of independence for weighted data, applying the second-order Rao-Scott correction. We carried out multivariate analyses using a logistic regression model on the discrete variables gender, Islands and age to identify the predominant factors influencing seropositivity. Finally, we looked at the non-linear relationship between age and seroprevalence using an ANOVA test, then a curve showing the predicted probabilities of having been infected was generated to better identify this relationship. A p -value of <0.05 was considered statistically significant and the 95% confidence intervals [CI95%] were calculated.

Results

Of the 1,150 participants approached, 1,120 were included in our study. Thirty individuals were not included mainly because of lack of signed consent forms for children or because they met at least one exclusion criteria. Among the 1,120 serum samples collected, 461 were from Efate Island, 258 from Espiritu Santo Island, 217 from Malekula Island and 184 from Tanna Island (Table 1). Ages ranged from 6 to 84 years-old with participants 18 years-old and younger comprising 29% of the cohort (compared to 46.3% according to the 2020 census). Females constituted 58% of the cohort (compared to 49.5% according to the 2020 census). In our cohort the average age for men is 37.17 (median 38.00 and IQR 39.00), whilst that for women is 33.36

(median 38.00 and IQR 32.00) (Sup Table 1). We did not observe any discrepancies between our target and the actual number of participants included across provinces.

Among the 1,120 serum samples, 417 had anti-DENV antibodies, 25 had anti-ZIKV antibodies and 108 had anti-RRV antibodies. Among the 25 positive ZIKV samples, 10 were also positive for DENV and among the 108 positive RRV samples one was also positive for CHIKV (Sup Table 2). Standardization to the population led to a global weighted seroprevalence of 34.0%, 3.2% and 8.6% for DENV, ZIKV and RRV respectively (Table 2). The immunity was very low or absent for JEV, YFV, WNV and CHIKV (Table 2). For malaria, the overall seroprevalence was 6.3% and 38.4% for *P. falciparum* and *P. vivax*, respectively, leading to 4.4% and 36.0% when adjusted to the population (Table 2).

When analyzing DENV seroprevalence in detail, significant differences between age classes were observed (Figure 1A), with the 6–18-years-old group less infected by DENV (11%) than all the others age groups (47 to 49%) ($p<0.001$). Multivariate logistic regression models (Supp Figure 2A) showed an independent association of age with DENV seroprevalence (OR 1.03 per year of increase in age, 95%CI [1.03-1.04], $p<0.001$), and that males were 1.39 times more likely to have been infected than females (95%CI [1.06 - 1.83], $p=0.023$). The predictive probability curve for DENV seropositivity showed that the relationship between seroprevalence and age was not linear (ANOVA test for non-linearity $p<0.001$). Indeed, the likelihood of DENV seropositivity increased from 6-years-old (approximately 3.0%) until it reached a plateau at 40 years-old (approximately 45%) (Sup Figure 2B). No significant differences in DENV seroprevalence were detected between islands (Figure 2A).

Regarding ZIKV, although the overall seroprevalence was low (3.2%) there were significant differences observed between islands. Malekula Island and Espiritu Santo Islands had higher ZIKV seroprevalence (6.50% and 5.80%, respectively) than Efate Island and Tanna Islands (0.80% and 0.50%, respectively) ($p=0.039$) (Figure 2A). No significant differences were detected according to gender or age class (Figure 1A). We did not carry out a multivariate analysis due to the low number of ZIKV positive serum samples ($n=25$).

Regarding RRV seroprevalence, we did not detect any significant differences according to islands (Figure 2A) or gender but there was a difference across age groups ($p=0.024$). We observed that RRV seroprevalence was present in all age groups, but that people aged between 45 and 64-years-old were more affected (15.0%) than other age groups (6.0 to 9.0%) (Figure 1A). For the 6-18 years-old group, RRV seroprevalence was 9% (i.e., 28 participants were positive). However, multivariate logistic regression models (considering the variables age, location and gender) (Supp figure 3A) showed that inhabitants of Tanna Island were 1.85 (95%CI [1.06-3.22], $p=0.033$) times less likely to have been infected compared to Efate Island. Interestingly, in this multivariate model age (considered here as a continuous variable) was not a variable associated with RRV seroprevalence (OR=1.01, 95%CI [1.00-1.01], $p=0.13$). The relationship between the likelihood of being RRV-positive and age is linear (ANOVA test, $p=0.42$), although there are some non-significant variations, the predictive probability curve remains stable at around a 10% chance (Supp Figure 3B).

When analyzing malaria seroprevalence in detail, no significant differences were noted according to gender regarding *P. falciparum* and *P. vivax* ($p=0.9$ and $p=0.8$ respectively). However, significant differences for *P. falciparum* were observed according to age ($p<0.001$),

with a number of infected people increasing with age (Figure 1B). In Tanna Island, anti-*P. falciparum* antibodies were found only in one participant aged of 51 (Figure 2B). In the other islands, anti-*P. falciparum* antibodies were found in 3.7%, 6.0% and 7.9% of the participants of Efate Island, Malekula Island and Espiritu Santo Island respectively. Significant differences were also observed for *P. vivax* according to the Islands ($p<0.001$). Seroprevalence was lower in Tanna Island at 21.0% (95%CI [17.0-24.0%]) compared to 38.0% (95%CI [26.0-52.0%]) in Malekula Island, 49.0% (95%CI [43.0-55.0%]) in Espiritu Santo Island and 36.0% (95%CI [28.0-44.0%]) in Efate Island (Figure 2B). Multivariate logistic regression model highlighted that for *P. falciparum*, relative to Efate Island seroprevalence was 2.69 times higher (95% CI [1.43-5.07]) in Espiritu Santo Island ($p=0.006$) and 8.33 times lower (95%CI [1.61-50.0]) in Tanna Island ($p=0.017$) (Supp Figure 4A). Age was a factor that has a minor influence on the seroprevalence of *P. falciparum*, with the odds of being infected increasing by 5% with every additional year of age (OR=1.05, 95%CI [1.04-1.07], $p<0.001$). The predictive probability increased linearly with age (non-linearity ANOVA test $p=0.095$), with the likelihood of having been infected reaching a maximum of 10% around 70 years-old (Supp Figure 4B). Espiritu Santo Island's inhabitants were 1.75 times (95%CI [1.29-2.38]) more likely to have been infected by *P. vivax* than Efate Island's inhabitants ($p=0.002$), while Tanna Island's inhabitants were 2.08 times (95%CI [1.56-2.78]) less likely to have been infected ($p=0.001$) (Supp Figure 4C). Age did not influence the risk of *P. vivax* seropositivity (OR=1.01, 95%CI [1.00-1.02], $p=0.088$). The odds of evidence of exposure were not increasing linearly with age (non-linearity ANOVA test $p=0.29$) and the predictive probability curve was stable at around 35% between the ages of 6 and 50, before rising to around 70% (Supp Figure 4D).

Discussion

In our study, we found that Vanuatu population has been exposed to substantial arboviruses and malaria transmission. These results are important given that the transmission and geographic distribution of arboviruses have not been historically well documented in Vanuatu and that recent heavy reliance on rapid diagnostic tests for malaria may have underestimated transmission [20].

Based on weighted data from a cohort representative of Vanuatu population, our study shows that DENV has been the major circulating arbovirus, with 34% of the population having antibodies against DENV. This number is consistent with epidemiological data, as DENV circulation has been reported in Vanuatu since the early 1970s, with all four DENV serotypes detected [9,15]. However, given the history of dengue fever in the Pacific [8,9,15], this seroprevalence seems relatively low compared to the seroprevalence rates in nearby islands such as Fiji (73%) [19] and Solomon Islands (56%) [21] or data from a recent study conducted on Espiritu Santo Island which reported an overall of DENV seroprevalence around 80% [22]. Discrepancies between studies may be explained by differences either in study design or in the test and antigens used: indirect commercial ELISA assay were used in these previous studies whereas our study used MIA relying on recombinant EDIII antigens [21,22]. Although we could not detect significant differences in DENV seroprevalence according to location, we have highlighted significant differences according to gender and age. Difference between male and female remains limited and can be explained by the study design, as gender was not used as a weighting factor. In our study, we observed an increase in dengue seroprevalence with age which peaks around age 40 at a maximum of 45%. For those aged approximately under 40, the likelihood of DENV previous exposure increases with age. Above the age of 40, most

exposures have already taken place and the likelihood of having been infected stays around 45%, albeit with possible geographical differences across provinces in the age or seroprevalence reached for this plateau. A recent study in Solomon Islands also highlighted a plateau for DENV seroprevalence around the age of 30 [21].

Our study also showed that Vanuatu population has been exposed to ZIKV, with a seroprevalence of 3.2%. Indeed, Vanuatu has been affected by the ZIKV outbreak that spread throughout the Pacific in 2013-2015 [23]. Given the important circulation of ZIKV in the region, this seroprevalence seems low. However, participants were sampled 10 years after the outbreak and recent studies highlighted that ZIKV immunity is waning with time [24]. This suggests that a higher proportion of people may have been exposed to ZIKV 10 years ago and that anti-EDIII ZIKV IgG have declined below the detection limit over time. This may explain the differences in ZIKV seroprevalence observed between our study and the one conducted in Espiritu Santo Island just two years after the outbreak, according to which seroprevalence reached 52% [22]. We also observed that inhabitants of Malekula Island and Espiritu Santo Island are the most affected by ZIKV, whereas those in Tanna Island show a significantly lower seropositivity. Although significant, these results are based on 25 positive sera out of 1,120 and should therefore be interpreted with caution.

We found evidence of previous RRV exposure suggesting low-level transmission in Vanuatu. Our data are in accordance with reports of RRV infections in travelers returning from Fiji and recent human seroprevalence studies in several PICTs suggest ongoing endemic transmission in the Pacific region including in neighboring Solomon Islands and Fiji Islands [21,25–28]. Furthermore neutralizing antibodies against RRV were detected in 28% to 100% of animal sera

sampled in Fiji Island [29]. Our study shows that 8.6% (n= 108) of the population is positive for anti-RRV IgG. Among the 108 individuals, only one had anti-CHIKV IgG, suggesting very low cross-reactivity between *Alphaviruses* antigens and thus reinforcing the possibility of low-level circulation of RRV. Furthermore, although we did not confirm our data with plaque-reduction neutralizing testing (PRNT), our RRV threshold interpretation was validated using both seroneutralisation and ELISA tests, thus strengthening our findings. Although we could not detect significant differences in RRV seroprevalence according to location or gender, we highlighted significant differences according to age, with the 45-64-years-old being more affected, which may be linked to the RRV outbreak that spread in the Pacific in 1979-1980 [30,31]. Furthermore, the probability of RRV previous exposition is constant across all ages, suggesting a silent circulation of RRV in Vanuatu, as observed in other PICTs [21,25,26,28].

Despite documented circulation of CHIKV across the Pacific [9] and a CHIKV seroprevalence of 8.1% found in a previous study conducted on Espiritu Santo Island [6], we detected only three positive samples for anti-CHIKV IgG. Our work highlights that the Vanuatu population seems immunologically naive to CHIKV despite the presence of potential vectors [32]. Our study also indicates that the Vanuatu population is naive for YFV, WNV and JEV.

Regarding malaria, our study found seroprevalence of 4.4% for *P. falciparum* and 36.0% for *P. vivax*. Few malaria seroprevalence studies have been conducted in Vanuatu with most limited to one or two Islands [33,34], making direct comparisons challenging. The high *P. vivax* seroprevalence observed in our study could be consistent with the increase in reported *P. vivax* cases in Vanuatu from 2021 to 2023. Historical data from 2009 reported seroprevalence on Tanna Island of 9.4% for *P. falciparum* and 12.6% for *P. vivax* [33]. Malaria elimination was

declared in Tafea province, including Tanna Island, in 2017 following three consecutive years without confirmed cases, and this status has been maintained through ongoing reports of zero cases. Despite this, our study identified a *P. vivax* seroprevalence of 21.0% on Tanna Island, with evidence of seroconversion amongst individuals aged 8 to 73 years old. As anti-malaria IgG mostly indicates past exposure rather than ongoing local transmission, these data must be interpreted with caution. They may indicate residual or previously undetected transmission of *P. vivax*, as malaria cases were confirmed in the last years in Vanuatu although this seropositivity may also likely reflect past exposure or relapse. In future surveillance studies, incorporating IgG avidity testing could serve as a complementary tool to differentiate recent from historical exposures by assessing antibody affinity maturation over time. Although this approach does not provide precise chronological dating of individual infections, relative avidity measurements could help refine population-level transmission dynamics [35]. For *P. falciparum*, 71 individuals were seropositive in our study, including one 51 years old female from Tanna Island, despite very few confirmed cases detected through passive surveillance since 2019. However, as travel was not reported in our study, we could not roll out, principally for Tanna Island, exposure in other islands or countries. Seroprevalence increased with age, and individuals over 40 years were more likely to have been exposed, suggesting that historical transmission was higher and that recent control efforts have reduced new exposures. Our study also shows that Tanna Island is the least affected Island with Espiritu Santo Island the most affected Island of those we surveyed, which is consistent with data from Vanuatu Ministry of Health Island.

Our study also suffers from limitations. Firstly, our study was conducted across the four main provinces of Vanuatu (Sanma, Malampa, Shefa, and Tafea), which together encompass 84.5%

of the total national population. Within these four target provinces, our sampling strategy covered areas representing 74.5% of their population. The remaining two provinces (Penama and Torba) were excluded due to severe logistical constraints. However, our study is the first, to our knowledge, to present weighted representative seroprevalence data regarding mosquito-borne diseases in Vanuatu four main islands/provinces. Secondly, our study suffers recruitment bias. Indeed, females were slightly overrepresented in our study, and the 18 years-old and younger underrepresented as compared to census data. Indeed, while weighting adjusts for discrepancies across age categories, it does not account for sex imbalances, as the sampling strategy was stratified solely by age and province. Inclusions were also conducted in the major towns, villages and main rural parts connected by main roads, thus excluding isolated tribes and rural areas. Although 1,150 participants were included in our study, we did not reach the target of 1,200 participants. Thirdly, travel history of the participants between islands was not reported. Therefore, we could not distinguish possible local exposure from infections potentially acquired elsewhere. There are two main hubs in Vanuatu: Port Vila/Efate (people are coming from the whole country) and Santo/Luganville (mostly for people from northern provinces as Torba, Penama and Malekula). Although, this information has less impact for DENV which has the higher seroprevalence rate in Vanuatu as compared to other arboviruses, it limits other arboviruses interpretation as we could not state where the participants got infected. This observation is also of importance for malaria interpretation particularly regarding Tanna Island for the only anti-*P. falciparum* IgG positive participant: we could not conclude if exposure occurred in Tanna, in another island or even overseas. Fourthly, we could not rule out some serological cross-reactivity for *Orthoflaviviruses* results (Sup Table 2). Indeed, in our assay DENV-serotype cross-reactivity remains, thus we could not provide DENV-serotype specific estimates for Vanuatu population

which is necessary to accurately estimate the dynamics of viral circulation and assess population-at-risk of (re)-infection. Also 10 sera were double DENV and ZIKV positive, for those one we could not rule out either multiple-reactivity or previous infections by both DENV and ZIKV as participants age were compatible with both sequential infections according to arboviruses past circulation in Vanuatu. Fifthly, we did not conduct PRNT which remains the gold-standard serological testing and would have ruled-out cross-reactivity. Although this may limit our interpretation, recent data [36] comparing multiplex serological arboviruses assay and PRNT highlighted that the SNAP-tagged EDIII and E2 antigens used in our study demonstrated improved specificity across multiple virus targets (including DENV, ZIKV, CHIKV and RRV). Although these results [36] need to be strengthened, it is emphasizing the importance of multiple antigens in the development of reliable serosurveillance tools in areas with overlapping arboviruses transmission. Finally, we did not collect clinical history of participants. This would have strengthened our data if serological positivity was associated with diagnosed infections. However, laboratory testing in Vanuatu regarding arboviruses is still scarce. This limitation is particularly relevant for RRV, for which epidemiological data in Vanuatu remain limited. Future prospective studies incorporating acute-phase clinical surveillance and RT-PCR/IgM testing will be necessary to better characterize recent and active RRV circulation in the region thus allowing a better timing of the infection, which cannot be ruled out by IgG alone.

Serological studies strengthen infectious disease surveillance as they often complement incomplete epidemiological and public health data. However, comparing seroprevalence results directly might be difficult due to differences in study design and testing techniques, considering their limits and the representativity or standardization of the data [37]. Yet,

serosurveys are more powerful when standardized and conducted using sensitive and specific tests. Our study used highly specific arboviruses antigens (EDIII domain for *Orthoflaviviruses* and E2 domain for *Alphaviruses*) with rigorous threshold validation as confirmed by the small number of cross-reacting sera obtained (ten positive sera for both DENV and ZIKV and one positive for both RRV and CHIKV). As we have chosen to favor specificity, we may have underestimated the overall seroprevalence, which could explain the discordance of our data compared to other studies where high DENV/ZIKV cross-reactivity were observed [21,22]. Although the arbovirus antigens we used had limited cross-reactivity between DENV and ZIKV, they do not fully remove cross-reactivity between DENV serotypes, thus limiting the performance of a more comprehensive analysis on the prevalence of each DENV serotypes. Furthermore, although chikungunya or dengue serotype antibodies are considered to be lifelong [38], antibody responses to malaria antigens wane over time [39], further confounding interpretation.

Our seroprevalence data complement the current epidemiological knowledge and show that the Vanuatu population has been exposed to substantial mosquito-borne diseases transmission with some local heterogeneity and potential age-related risk factors. However, surveillance systems do not reflect the actual disease burden in a population as they remain incomplete and tend to underestimate the extent of the outbreak [9]. Regarding *Orthoflaviviruses*, our results are consistent with surveillance reports and illustrate the disease burden caused by DENV. Regarding *Alphaviruses*, our data suggest that the geographical range of RRV in the PICTs is larger than previously documented. Notably, RRV diagnostics are not available in Vanuatu (and most PICTs). As symptoms overlap with DENV, ZIKV, CHIKV or other diseases not transmitted by arthropods such as leptospirosis, low-level RRV circulation likely

occurred undetected in Vanuatu. This highlights the need to consider the implementation of RRV diagnosis in Vanuatu and the need for studies focusing on RRV local transmission to identify local reservoirs and vectors. Furthermore, as Vanuatu joined the World Mosquito Program, *Wolbachia*-carrying *Aedes aegypti* were released in Port-Vila, Efate Island, thus potentially recently limiting DENV, CHIKV and ZIKV circulation. Our study, conducted just after *Wolbachia* releases, will provide a unique baseline to assess the effectiveness of this strategy in the future. Finally, malaria data generated in our study highlights the need to strengthen the fight against *P. vivax* whilst remaining vigilant for *P. falciparum*. With the latest framework and in consideration of recent malaria surges, the Vanuatu Ministry of Health now aims to achieve country-wide elimination of malaria by 2031.

In conclusion, seroprevalence studies provide important reference data that can be used to monitor the evolution of seroconversion at the population scale, retrospectively evaluate the effectiveness of control measures, make decisions and assess the risk for future epidemics. Conducting similar sero-epidemiological approaches in other PICTs will be useful to generate a regional picture of mosquito-borne diseases circulation and improve our knowledge of pathogen transmission forecasts in the Pacific.

Conflict of Interest

Authors declare no competing interests.

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Ethical Approval Statement

The current study was conducted in compliance with the Declaration of Helsinki principles. Samples came from participants who gave their written informed consent as part of this study. A prospective study protocol was set up ahead of study implementation. This study protocol was approved by the Institutional Review Board at the Institut Pasteur (n° IRB2021-02 PIANO, July 23rd, 2021) and by the Vanuatu Health Research Ethics (October 18th, 2021). The study was recorded on clinicaltrials.gov (NCT05153018).

Author contributions

MDR, CI and OOC conceived the study. MDR led the funding applications. Consent and questionnaire tools were designed by MDR, WD, LV, and CI. Inclusions and samples collection were implemented by LV, WD, AK, AFG. Supervision of the inclusions was performed by CD, TK, PG and WD. KH performed the experiments with the support from DH and LG. KH implemented the statistical analyses under the supervision of JV, CI and MW. KH wrote the scientific manuscript with the support of MDR and with input from CI, JV, MW, TK, PG, AFG, and OOC. All authors reviewed the manuscript and approved the final version prior to submission.

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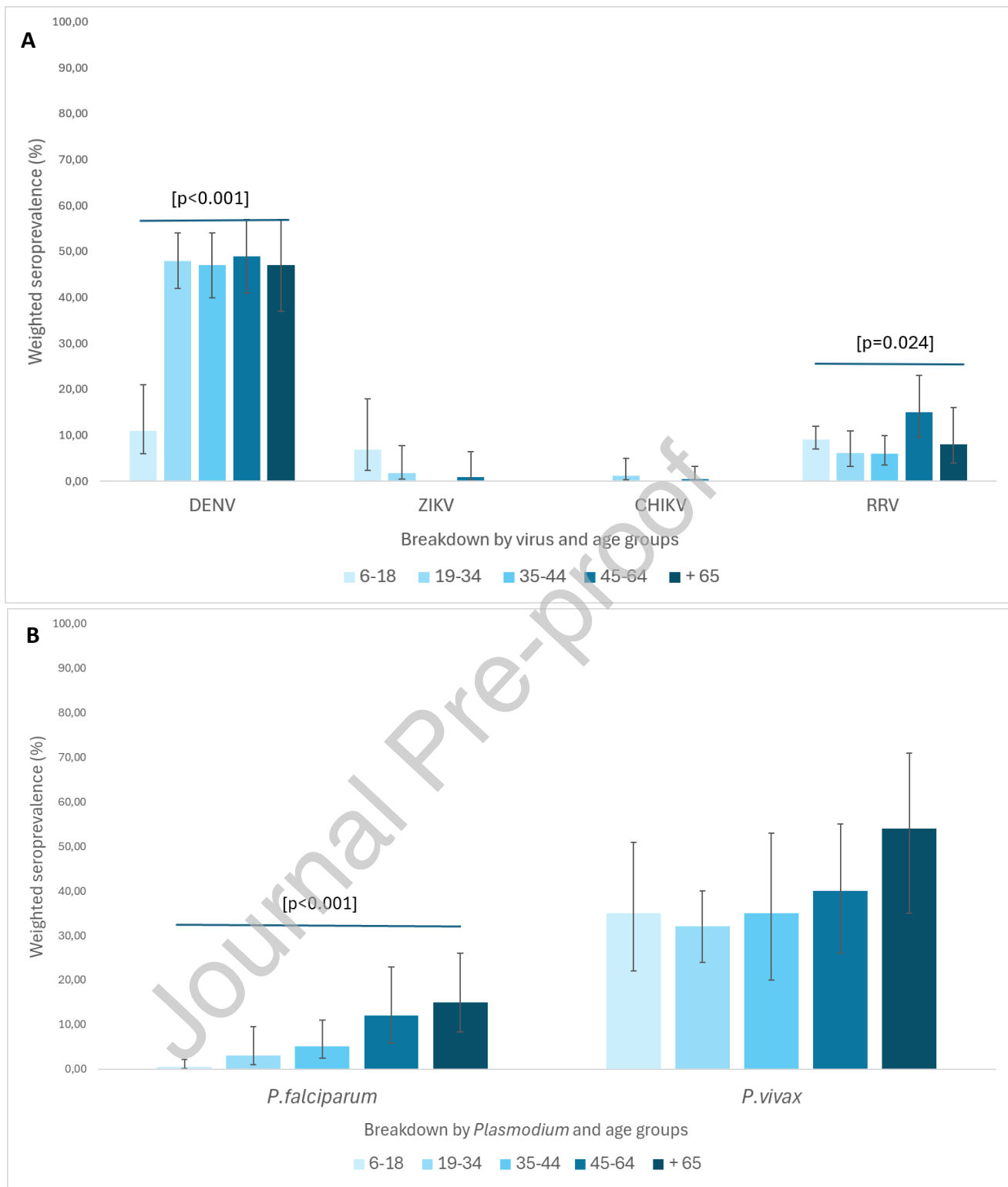


Figure 1: Weighted arboviruses and malaria seroprevalence by Vanuatu's age groups.

Bars represent weighted seroprevalence rates (% with 95% confidence intervals) across five age groups for: A) dengue virus (DENV), Zika virus (ZIKV), chikungunya virus (CHIKV), Ross River virus (RRV), and B) *P. falciparum* and *P. vivax*. Seroprevalence rates were compared

using Pearson's Chi-square test with Rao & Scott adjustment, and significant p-values are shown above the relevant comparisons.

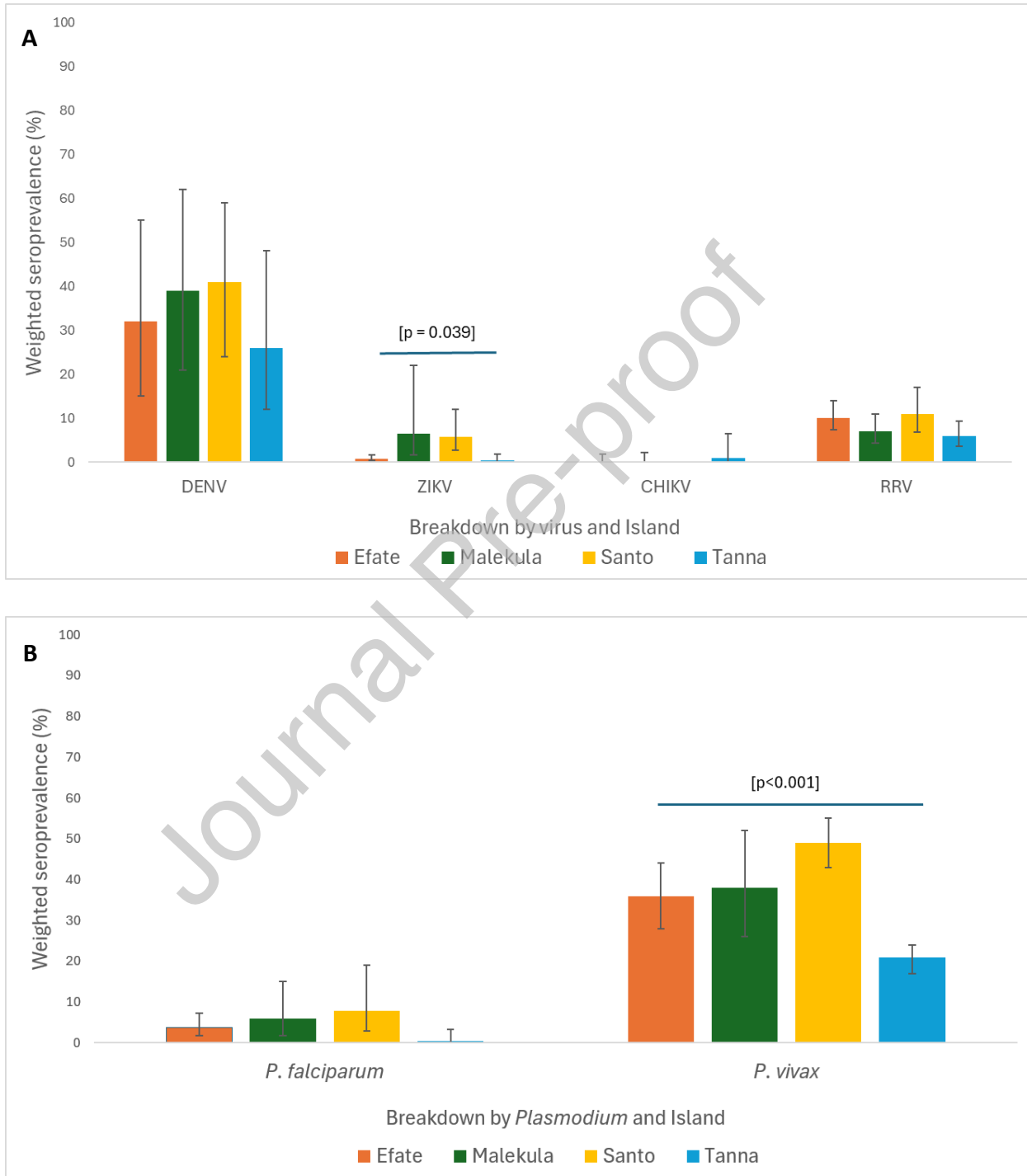


Figure 2: Weighted arboviruses and malaria seroprevalence by Vanuatu's Islands

Bars represent weighted seroprevalence rates (% with 95% confidence intervals) across the four Islands for: A) dengue virus (DENV), Zika virus (ZIKV), chikungunya virus (CHIKV) and Ross River virus (RRV), and B) *P. falciparum* and *P. vivax*: orange Efate Island (Shefa province) (n=461), green for Malekula Island (Malampa province) (n=217), yellow for Espiritu Santo Island (Sanma province) (n=258), and blue for Tanna Island (Tafea province) (n=184). Statistical comparisons between Islands were performed using Pearson's Chi-square test with Rao & Scott adjustment, and significant *p*-values are shown above the statistically significant comparisons.

Table 1: Number of serum samples per Island and age group, Vanuatu, 2023

	Age group					
Island (Province)	6-18	19-34	35-44	45-64	65+	All
Efate Island (Shefa)	136	93	64	127	41	461
Espiritu Santo Island (Sanma)	48	83	55	54	18	258
Malekula Island (Malampa)	56	29	43	57	32	217
Tanna Island (Tafea)	91	29	26	32	6	184
Total	331	234	188	270	97	1120
% collected per age group	29%	21%	17%	24%	9%	100%

Table 2: Adjusted arboviruses and malaria seroprevalence, general Vanuatu population, 6-84 years-old, Vanuatu, 2023

	Number of positive IgG samples	Overall seroprevalence, weighted (%)	95% confidence interval
DENV	417	34.0	28.0 – 41.0

WNV	2	0.4	0.05 – 2.8
YFV	0	0	0
JEV	0	0	0
ZIKV	25	3.2	1.3 – 7.5
CHIKV	3	0.4	0.1 – 1.4
RRV	108	8.6	7.1 – 11.0
<i>P. falciparum</i>	71	4.4	2.8 – 7.0
<i>P. vivax</i>	430	36.0	29.0 – 43.0

Declaration of Interest Statement

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for this journal and was not involved in the editorial review or the decision to publish this article.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical abstract

A multiplex serological survey highlights differences in population immunity to mosquito-borne diseases in Vanuatu, Pacific region, 2023.

