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Harriet L S Lawford

h.lawford@uq.edu.au

University of Queensland

Grizelda Mokoia

Department of Health

Patricia Tatui

Department of Health

Misiona Nicolas

Department of Health

Andy Manu

Department of Health

Amanda Murphy

James Cook University

Tonia Marquardt

Queensland Health

Leanne J Robinson

Burnet Institute

Fiona Angrisano

Burnet Institute

Colleen L Lau

University of Queensland

Adam T Craig

University of Queensland

Article

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Seroprevalence of neglected tropical diseases, arboviruses, vaccine-preventable diseases, and enteric protozoa from a national multi-pathogen serosurvey in Niue

Running title: Multi-pathogen serosurveillance in Niue

Harriet L S Lawford^{1*}, Grizelda Mokoia², Patricia Tatui², Misiona Nicolas², Andy Manu², Amanda Murphy³, Tonia Marquardt⁴, Leanne J Robinson⁵, Fiona Angrisano⁵, Colleen L Lau¹, Adam T Craig¹

1. The University of Queensland, Frazer Institute, Faculty of Health, Medicine, and Behavioural Sciences, Brisbane, QLD, Australia
2. Department of Health, Alofi, Niue
3. James Cook University, Australian Institute of Tropical Health and Medicine, Cairns, QLD, Australia
4. Queensland Health, Brisbane, QLD, Australia
5. Burnet Institute, Melbourne, VIC, Australia

* Corresponding author

Email: h.lawford@uq.edu.au

ORCID: 0000-0002-9699-8201

Address: Frazer Institute, Faculty of Health, Medicine and Behavioural Sciences, The University of Queensland, Herston, QLD 4029, Australia

Biography: Dr Harriet Lawford is a field epidemiologist at The University of Queensland specialising in infectious disease surveillance and elimination. Her main interests include the epidemiology of lymphatic filariasis and malaria, integrated serosurveillance, and operational research supporting national and regional elimination efforts.

ABSTRACT

Integrated serosurveillance (ISS), using multiplex bead assays to measure antibodies to multiple pathogens, can provide insights into population immunity and infection. We estimated seroprevalence for neglected tropical diseases (NTDs), arboviruses, vaccine-preventable diseases, and enteric protozoa in Niue through a cross-sectional survey embedded within a WHO STEPwise survey in February-March 2025. Dried blood spots from participants aged ≥ 3 years were tested for 26 pathogen-specific antibodies. Among 750 participants (median age 37 years; 53% female), NTD seroprevalence was low (0% for yaws to 2.7% for cysticercosis). Arbovirus seroprevalence ranged from 3.1% (Zika) to 35.1% (dengue) and increased with age ($p < 0.05$). Measles and rubella seroprevalence exceeded 98%, while diphtheria and tetanus protection declined with age ($p \leq 0.03$). *Cryptosporidium* seroprevalence (68.7%) increased with age ($p < 0.001$), while giardiasis remained low (4.1%). Findings demonstrate the utility of ISS to generate policy-relevant surveillance data and support targeted vaccination, arbovirus surveillance, and elimination strategies in Pacific Island settings.

Keywords: integrated serosurveillance; multiplex bead assays; infectious diseases; elimination; Pacific Island Countries and Territories, Niue.

INTRODUCTION

Serological surveillance (or serosurveillance), the testing of blood samples to monitor the prevalence of antibodies (Ab) to pathogen-specific antigens (Ag), provides insights into population-level immunity to vaccine-preventable diseases (VPDs) and other infectious diseases. Depending on the pathogen, the presence of Ab (termed seropositivity) may reflect immunity from vaccination or ongoing/chronic infection. Integrated serosurveillance (ISS) is the simultaneous estimation of prevalence of Ab to multiple pathogens[1] and offers an efficient means to monitor multiple infectious diseases concurrently. ISS can be used by national and regional public health programs to identify populations with waning immunity and/or immunity gaps for VPDs, as well as monitor the prevalence of infectious diseases nearing elimination, or that are assumed to be eliminated.[2]

Neglected tropical diseases (NTDs) are a group of 21 infectious diseases, mainly affecting populations in tropical and subtropical regions, that have historically received less attention and funding than other illnesses. In the *Roadmap for Neglected Tropical Diseases 2021–2030*,[3] WHO outlined ambitious goals to eliminate NTDs, with targets set for elimination of at least one NTD in 100 countries by 2030, and complete global eradication of two NTDs (yaws and dracunculiasis [Guinea worm disease]). Similarly, WHO's *Immunization Agenda 2030* has regional disease-specific elimination goals for 2030, including measles and rubella, with elimination defined as “the absence of endemic transmission in a defined geographical area for 12 months with well-performing surveillance”.[4, 5]

Both the NTD Roadmap 2021-2030[3] and the Immunization Agenda 2030[4] have highlighted the importance of sustained surveillance, particularly ISS, to achieve and monitor elimination goals.

Multiplex bead-based assays (MBA) are a bead based assay capable of detecting Ab responses to multiple analytes simultaneously from a single dried blood spot (DBS),[6] supporting a multi-pathogen surveillance approach. MBA for population-level multi-pathogen surveillance can be both cost-effective and less resource intensive compared to time-consuming single-analyte assays, while concurrently

estimating patterns of infection and immunity to inform holistic, evidence-based public health action.[7] MBAs have been used for surveillance of eliminated and near-eliminated diseases, such as lymphatic filariasis (LF),[8] helminth infections, particularly *S. stercoralis* infection,[9] the zoonotic NTD *Taenia solium*,[10] and enteric protozoa including *Giardia lamblia* and *Cryptosporidium parvum*. [11] For VPDs, serosurveys are considered the gold standard for measuring population immunity; MBAs can be leveraged to estimate population immunity to multiple VPDs simultaneously, enabling identification of disparities in Ab seroprevalence and vaccine uptake.[2]

Niue, a small (261 km²) self-governing island in the South Pacific, is one of the leaders in disease elimination in the Pacific region. Niue's ambitious "Cure-a-Country", launched in 2019, has placed the country on track to become the first in the world to eliminate viral hepatitis (specifically hepatitis B and C) as a public health problem.[12] Niue has consistently achieved high vaccination coverage, with >95% of the target population covered for routine childhood vaccines.[13] Their vaccination schedule is generally aligned with that of New Zealand including two measles-mumps-rubella (MMR) and four diphtheria-tetanus-pertussis (DTP) vaccines by the age of five with DTP boosters at 11, 45 and 65 years old. Whilst occasional cases are reported, no significant outbreaks of VPDs, other than SARS CoV-2, have been reported in the preceding 10 years. Further, Niue was validated by the World Health Organization (WHO) as having successfully LF as a public health problem in 2016,[14] with several other NTDs, including trachoma and leprosy, considered eliminated.

Post-validation surveillance (PVS) is a surveillance approach strongly encouraged by WHO once a country has eliminated LF as a public health problem.[15] The term *elimination as a public health problem* refers to reducing disease burden to below a WHO-defined threshold within a specific geographic area (requiring continued monitoring to ensure prevalence levels remain below the threshold). In contrast, *elimination of transmission* refers to zero incidence in a defined geographic area (requiring ongoing measures to prevent reintroduction) and *eradication* which denotes the permanent global reduction of infection to zero, after which intervention measures are no longer needed.[16] Thus, the aim

of PVS is to ensure that resurgence of LF has not occurred and prevalence has remained below target thresholds.

In 2025, LF-PVS in Niue was embedded into a planned and funded national WHO STEPwise non-communicable disease (NCD) risk factor (STEPs) survey. In addition to asking NCD-related lifestyle, diet, and behavioural questions, finger-prick blood samples were collected from participants.[17] Alongside testing blood samples for glucose levels, cholesterol levels for the STEPs survey, and LF Ag for PVS, DBS samples were prepared for MBAs. The study protocol[17] and LF-PVS results[18] have been published separately.

This current study aimed to estimate seroprevalence of 17 diseases, including NTDs (LF, yaws, trachoma, taeniasis, cysticercosis, strongyloidiasis), arboviruses (dengue, chikungunya, Zika), VPDs (SARS CoV-2, measles, rubella, diphtheria, tetanus, pertussis), and enteric protozoa (cryptosporidiosis, giardiasis) to provide insights to inform public health strategies to prevent, control, and eliminate infectious diseases in Niue. Our specific objectives were to estimate seroprevalence by (i) sex (males vs. females); (ii) age group (<20 years, 20-49 years, ≥50 years); (iii) recruitment site (Alofi vs other villages); and (iv) demographic associations for seropositivity.

METHODS

Ethics approval

The study was approved by the Research Committee of the Niue Department of Health (5 August 2024) and ratified by the UQ Human Research Ethics Committee (approval reference 2024/HE001375; 8 August 2024), and the Alfred Hospital Ethics Committee, Melbourne, Australia (approval reference 317/25).

Written informed consent was obtained from all participants, with parent or guardian informed consent for participants aged <18 years. All personal identifiers were removed prior to analysis in accordance

with the United States Department of Health and Human Services' Health Insurance Portability and Accountability Act Privacy Rule. The study was conducted in accordance with relevant ethical guidelines and regulations, including the Declaration of Helsinki issued by the World Medical Association and the International Ethical Guidelines for Health-related Research Involving Humans published by the Council for International Organizations of Medical Sciences.

Study setting

Niue, one of the largest raised coral atolls in the world, is located approximately 660 km southeast of Samoa and 2,400 km northeast of New Zealand.[18, 19] The island has a population of ~ 1,600 people (about 1,300 of whom are on the island at any one time).[18] Many people with Niuean heritage live in Aotearoa New Zealand (30,867 Niueans recorded in the 2018 census), Australia (4,958 Niueans recorded in the 2016 census), and other Pacific Islands.[19] The island is divided into 14 administrative areas, each with a village centre (Figure 1). Most of the population live along or close to the coast. Alofi, the capital and only town of Niue, is divided into two jurisdictions: Alofi North and Alofi South.

Study design

The study protocol was co-designed with the Niue Department of Health (DOH) with input from the WHO STEPs team and has been described in detail elsewhere.[17] In brief, the LF-PVS was conducted as a population census of individuals aged ≥ 3 years integrated with a planned national population-based STEPs survey. The survey was conducted from 24th February to 4th April 2025. The STEPs survey's target population were all individuals aged 16 to 69 years with surveying conducted in community and church halls across the 14 villages in Niue. To expand the age range of LF-PVS to include younger (3 to 16 years) and older (> 69 years) participants, convenience community-based sampling was conducted at the country's only primary and high school, the morning market, the bank waiting area, workplaces, churches, and the outpatient clinic at Niue Foou Hospital.

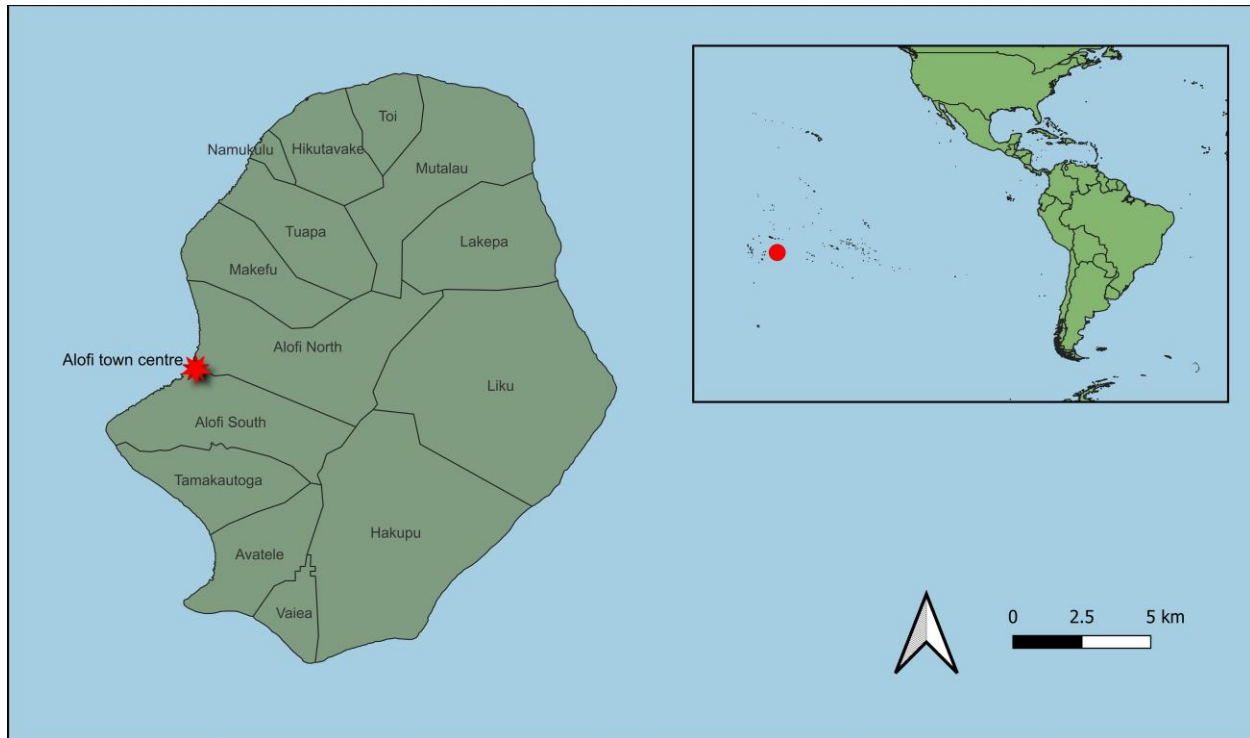


Figure 1: Map of Niue showing administrative regions, the capital (Alofi) and Niue's location in the Pacific Ocean (insert). Alofi town centre indicated by red star.

Data and Sample Collection

Data and sample collection have been described elsewhere.[18] Each participant was allocated a unique QR-coded identifier, which was affixed to all study materials (consent forms, questionnaires, blood collection tubes, LF Ag tests [Bioline™ Filariasis Test Strip (FTS); Abbott, Scarborough, ME], and dried blood spots [DBS]) to support linkage and tracking. Upon giving written informed consent, participants provided demographic information (age, sex, country of birth, village of residence, occupation), had at least 300µL of finger-prick blood collected into heparin-coated BD Microtainers® Blood Collection Tubes for LF testing and DBS preparation, and then completed questions on international travel history. STEPs data were captured in a purpose-built tablet-based data collection tool. LF-PVS data were collected using EpiCollect5 (version 76.1.0; <https://five.epicollect.net>). Data were merged using Stata statistical software (StataCorp, Version 19.5, College Station, TX).

Multiplex bead assays

Details on definitions and interpretation of seropositivity can be found in the Appendix. The chemical coupling of Ags to MagPlex microspheres (beads) (Luminex Corp., Austin, TX) were provided by the US Center for Disease Control and Burnet Institute. Briefly, DBS were eluted into 96-well plates, diluted to a final concentration of 1:400 and assayed for Ab on an MBA containing 26 Ag-coupled beads from 17 pathogens grouped into five disease categories: NTDs (LF [*Bm14* and *Wb123*], trachoma [*Pgp3*], yaws [*Rp17* and *TmpA*], taeniasis [*Es33*], cysticercosis [*T24H*], and strongyloidiasis [*SsIR*]), arboviruses (dengue virus [DENV] 1–4 [*Ns1-1*, *Ns1-2*, *Ns1-3*, *Ns1-4*], chikungunya [*Chik E1*], and Zika [*Zika NS1*]), VPDs (tetanus [*Tet*], diphtheria [*Dip*], measles [*MeV*], rubella [*RuV*] pertussis [*Pt*], SARS CoV-2 [*Spike*, *N*, *RBD591*], and enteric protozoa (cryptosporidiosis [*Cp17* or *Cp23*] and giardiasis [*Vsp3* or *Vsp5*]).

For trachoma, *Pgp3* Ab seropositivity reflects cumulative exposure to *Chlamydia trachomatis* and is considered an early indicator of transmission but cannot distinguish ocular infection from urogenital exposure or perinatal transmission[20] thus seroprevalence estimates for these pathogens were restricted to <14-year-olds only. For yaws, *Rp17* Ab seropositivity is considered indicative of past infection, whilst *TmpA* Ab are indicative of recent infection. Dual positivity suggests current or very recent yaws, and for the purpose of this paper, will be considered the main measure of yaws infection. Similarly, MBAs cannot differentiate yaws infection from syphilis, thus seropositivity estimates for yaws were also limited to ≤14-year-olds only.

Sample plates were read on a Bio-Plex 200 instrument (Bio-Rad, Hercules, CA). For each Ag, median fluorescence intensity minus background (MFI-bg) signal was used to determine seropositivity. Thresholds for seropositivity were determined using one of three approaches depending on the availability of reference samples: (1) the mean plus three standard deviations (SD) of the MFI of known negative sera; (2) receiver operating characteristic (ROC)-derived MFI cut-offs based on panels of known positive and negative samples, where positives were defined by PCR or reference serological assays (e.g.,

Treponema pallidum particle agglutination/rapid plasma reagin) and negatives included sera from individuals with no evidence of infection, such as non-travellers from non-endemic settings; or (3) standard curves generated using WHO International reference serum standards. Using these standards, concentrations were calculated using linear regression fit to log-transformed values from dilution series with seroprotective thresholds international units per millilitre (IU) values. For VPDs, thresholds corresponding to partial protection (>0.01 to <0.1 IU/mL), full protection (≥ 0.1 to <1.0 IU/mL), and maximum (≥ 1 IU/mL) antibody levels were calculated for diphtheria and tetanus. Thresholds for defining seropositivity were measles >153 mIU/mL and rubella >9.36 IU/mL. As there is internationally recognised seroprotection cutoff for pertussis, a threshold of 0.1 IU/mL corresponding to the lower limit of detections of the assay was used to indicate detectable antibody presence rather than confirmed protective immunity.

Statistical analysis

Data were analysed using Stata statistical software (StataCorp, Version 19.5, College Station, TX). Summary statistics with 95% confidence intervals (95% CI) for Ab seroprevalence were calculated for age groups (<20 years, 20–49 years, and >50 years), sex (male or female), and recruitment site (Alofi or other villages). Multivariable logistic regression was used to assess associations between age, sex, recruitment site and seropositivity for each Ab. Age group and sex were included *a priori* in all multivariable models because of biological relevance. Interaction terms were assessed and retained if statistically significant ($p < 0.05$). Estimated seroprevalence (presented as percentages) was calculated using the ‘*margins*’ command in Stata following multivariable logistic regression models. Marginal predicted probabilities and 95% CIs of seropositivity (expressed as percentages) were calculated for each covariate category.

RESULTS

Study population demographics

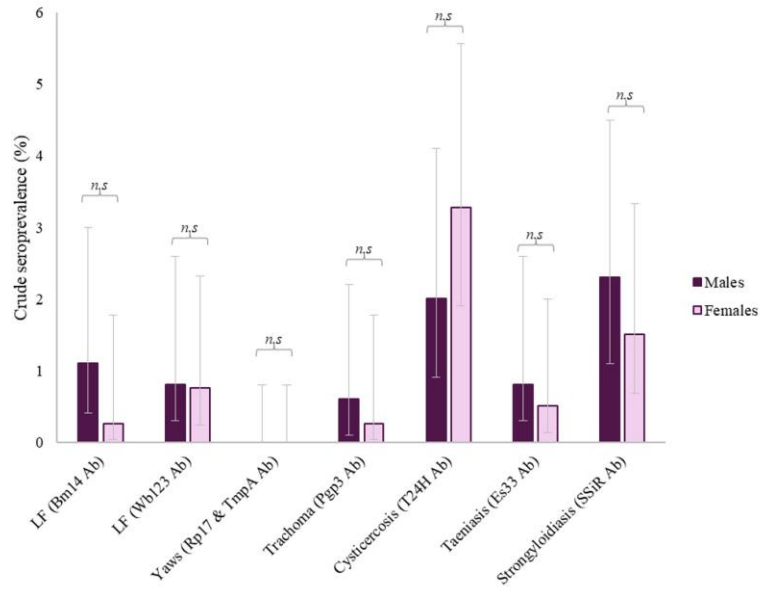
Of 898 participants recruited, 755 (84.1%) had DBS collected of whom 750 (99.3%) were matched to demographic records. The median age of participants was 37 years (range: 3-93 years) and 52.9% (n=397) were female. Overall, 32.5% (n=244) of participants were aged <20 years, 35.5% (n=266) 20-49 years, and 32.0% (n=240) ≥50 years. Twenty-eight percent (n=213) of participants were recruited from the capital, Alofi, with the remaining participants recruited from other villages (Table 1).

Seroprevalence estimates varied substantially across pathogens and are summarised for all Ab stratified by age and sex (Supplementary Table 1) and recruitment site (Supplementary Table 2). Similar variations were seen in demographic associations with seropositivity (Supplementary Tables 3a-e). Results are summarised by disease groups below.

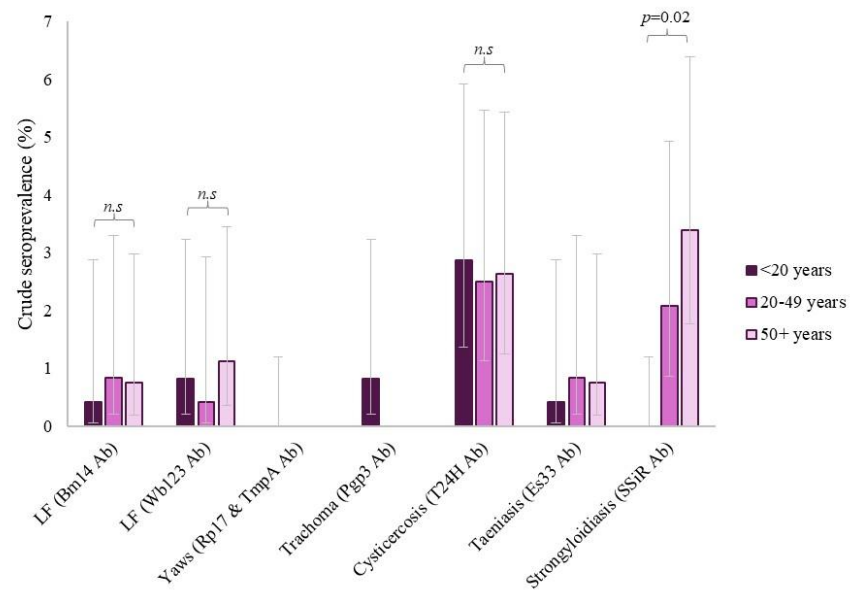
Neglected tropical diseases

Overall, Ab seroprevalence ranged from 0% seropositivity for yaws to 2.7% for cysticercosis. For each NTD, no significant difference was seen in seroprevalence when stratified by sex or age group, except for seropositivity for strongyloidiasis ($p<0.001$) which showed significantly higher seropositivity as age increased (<20 years=0%, 20-49 years=2.1%, ≥50 years=3.4%; $p=0.02$) (Figure 2, Panel A). No significant associations were seen between seroprevalence of individual NTDs and age group, sex, or recruitment site (Supplementary Table 3a).

A)



B)



C)

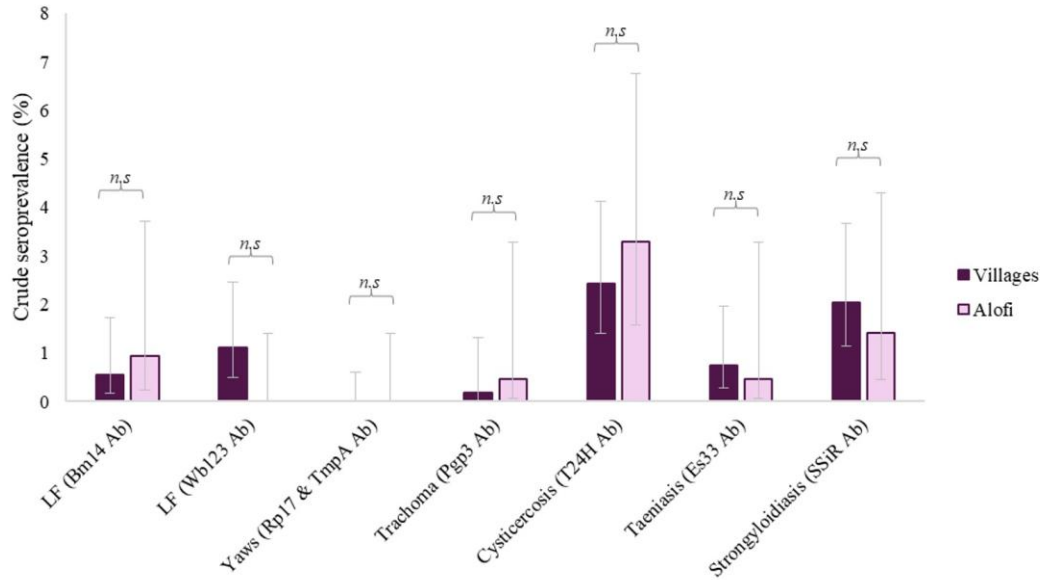


Figure 2: Seroprevalence of antibodies to neglected tropical diseases in Niue in 2025 by sex (Panel A: males represented in dark purple bars and females in light purple bars), age (Panel B: <20 years [dark purple bar], 20-49 years [purple bar], ≥50 years [light purple bar]), and recruitment site (Panel C: Alofi represented by the light purple bar and other villages represented by the dark purple bar). Analyses for trachoma and yaws restricted to participants aged ≤14 years (total=165 participants). Significance tested using univariable logistic regression. n.s = “non-significant”.

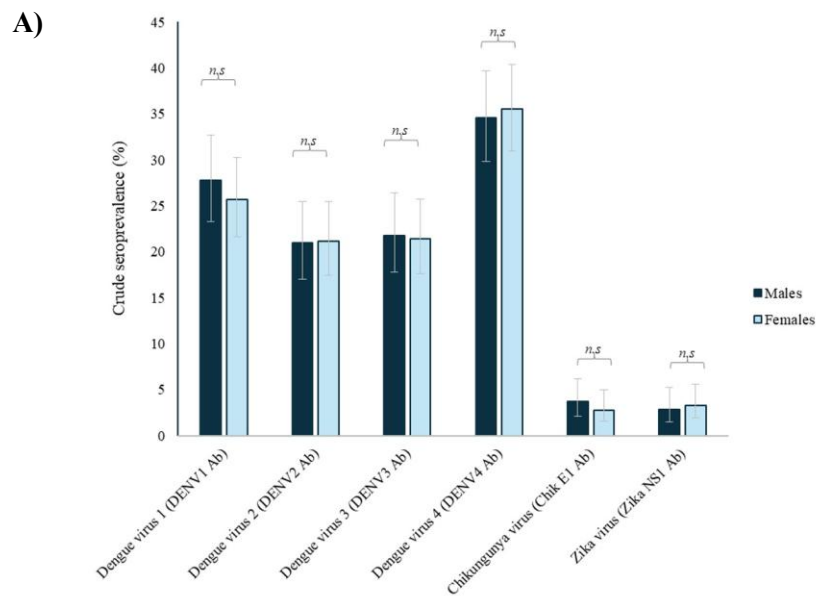
Arboviruses

Arbovirus seroprevalence ranged from 3.1% for Zika to 35.1% for *DENV-4*. Seroprevalence for all dengue serotypes were significantly higher as age group increased ($p<0.001$; Supplementary Figure 1). For chikungunya ($p=0.01$) and Zika ($p<0.001$), the highest seroprevalence was seen among 20-49-year-olds and the lowest among ≥50-year-olds (Figure 3, Panel B; Supplementary Table 1 and Supplementary Figure 2). Significantly higher seroprevalence for *DENV-2* ($p=0.01$), *DENV-3* ($p=0.02$), and Zika ($p<0.001$) were seen in the capital Alofi compared to other villages (Figure 3, Panel C and Supplementary Table 2).

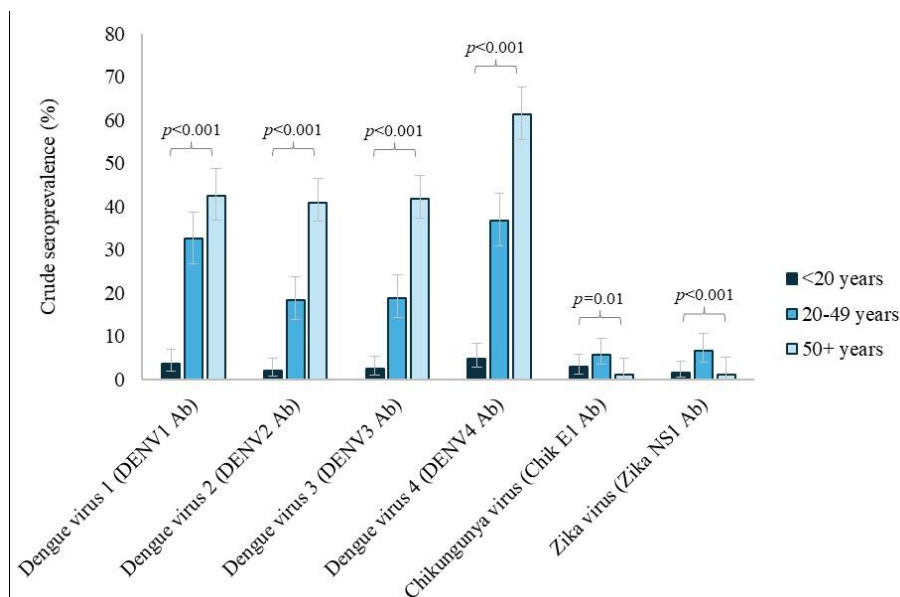
Compared to <20-year-olds, participants aged ≥50 years and 20-49 years had significantly higher aOR for seropositivity to all dengue serotypes ($p<0.001$). Participants aged 20-49 years had significantly higher

aOR (4.7 [1.5-14.6]; $p=0.007$) for Zika seropositivity compared to <20-year-olds. For all arboviruses except chikungunya, participants had significantly higher aOR for seropositivity if they were recruited from Alofi compared to other villages (Supplementary Table 3b).

A strong and consistent age gradient in seropositivity for *DENV-1*, *DENV-2*, *DENV-3* was seen, with markedly higher predicted seroprevalence among adults compared with children; seroprevalence was lowest among children aged 3-9 years in villages and highest among adults aged ≥ 50 years in Alofi. Zika seroprevalence was lower overall but showed higher predicted seroprevalence in Alofi and among adults aged 20-49 years (Supplementary Table 4a).



B)



C)

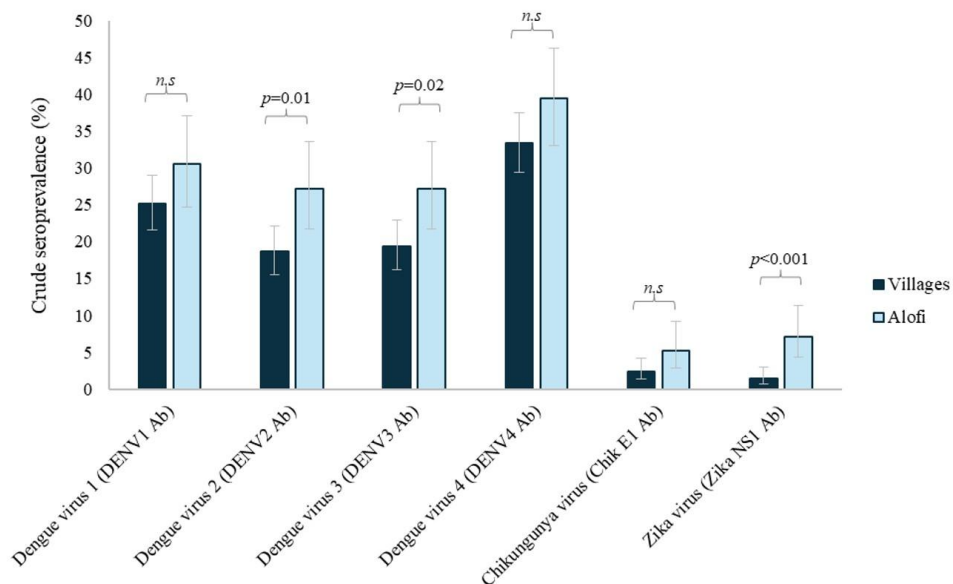


Figure 3: Seroprevalence of antibodies to arboviruses in Niue in 2025 by sex (Panel A: males represented in dark blue bars and females in light purple bars), age (Panel B: <20 years [dark blue bar], 20-49 years [blue bar], ≥50 years [light blue bar]), and recruitment site (Panel C: Alofi represented by the light blue bar and other villages represented by the dark blue bar). Significance tested using univariable logistic regression. n.s = “non-significant”.

Seroprevalence to arboviruses by 10-year age groups

When stratified by 10-year age groups (Table 1), significant differences in seroprevalence were seen for all arboviruses except chikungunya. No participant aged 3-9 years tested positive for any arbovirus. For dengue, clear increases with age were seen with the highest seroprevalence seen among 60-69-year-olds for *DENV-1* (46.4%), *DENV-2* (47.3%), and *DENV-3* (48.2%), and among 50-59-year-olds for *DENV-4* (65.1%). The lowest seroprevalence for all dengue serotypes (aside from <10-year-olds who did not test positive for any arbovirus) was among 10-19-year-olds. For Zika, the highest seroprevalence was seen among 20-29-year-olds (10.1%) with no participants aged 3-9 years or ≥ 70 years testing seropositive. Lastly, for chikungunya, the highest seroprevalence was seen among 30-39-year-olds (8.5%), with no participants aged 3-9 years, 50-59 years, or ≥ 70 years testing seropositive.

256 **Table 1: Seroprevalence of arboviruses, stratified by 10-year age groups, Niue 2025. P-value for age group obtained using Wald χ^2 test (df=7).**

Disease (antigen)	Age group (years)																	P-value	
	Total (n=750)		3-9 (n=70)		10-19 (n=174)		20-29 (n=69)		30-39 (n=82)		40-49 (n=89)		50-59 (n=109)		60-69 (n=110)		70+ (n=47)		
	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N		% (95% CI)
Seropositive for VPDs																			
SARS CoV2 (RBD591)	734	97.9	59	84.3	172	98.9	69	100	81	98.8	89	100	109	100	108	98.2	47	100	<0.001
		96.5-98.7				73.8-91.1				95.5-99.7				-				91.8-99.8	
SARS CoV2 (Spike)	746	99.5	69	98.6	174	100	69	100	81	98.8	89	100	109	100	108	98.2	47	100	0.94
		98.6-99.8				90.5-98.8				-				-				91.8-99.8	
SARS CoV2 (N)	465	62	48	68.6	127	72.8	39	56.5	49	59.8	59	66.3	62	56.9	62	56.4	19	40.4	0.002
		58.5-65.4				56.8-78.3				65.9-79.1				44.7-67.7				48.8-69.8	
Measles virus 0.01 IU/mL (MeV)	741	98.8	70	100	172	98.9	68	98.6	77	93.9	88	98.9	109	100	110	100	47	100	0.11
		97.7-99.4				-				95.5-99.7				90.4-99.8				86.2-97.4	
Rubella virus 0.01 IU/mL (RuV)	736	98.1	70	100	173	99.4	68	98.6	78	95.1	89	100	105	96.3	109	99.1	44	93.6	0.18
		96.9-98.9				-				96.0-99.9				90.4-99.8				87.7-98.2	
Tetanus toxoid 0.01 IU/mL (Tet)	716	95.5	68	97.1	171	98.3	67	97.1	77	93.9	84	94.4	104	95.4	102	92.7	43	91.5	0.38
		93.7-96.7				89.3-99.3				94.8-99.4				89.1-99.3				86.2-97.4	
Tetanus toxoid 0.1 IU/mL (Tet)	554	73.9	53	75.7	142	81.6	55	79.7	59	72	66	74.2	70	64.2	74	67.3	35	74.5	0.045
		70.6-76.9				64.3-84.4				75.1-86.7				68.6-87.6				61.3-80.6	
Diphtheria toxoid 0.01/mL (Dip)	509	67.9	63	90	161	92.5	57	82.6	56	68.3	52	58.4	47	43.1	46	41.8	27	57.5	<0.001
		64.4-71.1				80.5-95.2				87.6-95.6				71.8-89.9				57.5-77.4	
Diphtheria toxoid 0.1/mL (Dip)	248	33.1	39	55.7	106	60.9	31	44.9	27	32.9	14	15.7	11	10.1	13	11.8	7	14.9	<0.001
		29.8-36.5				44.0-66.9				53.5-67.9				33.7-56.8				23.6-43.8	
Pertussis 5mIU/mL (Pt)	750	100	70	100	174	100	69	100	82	100	89	100	109	100	110	100	47	100	-
		-				-				-				-				-	

Vaccine-preventable diseases

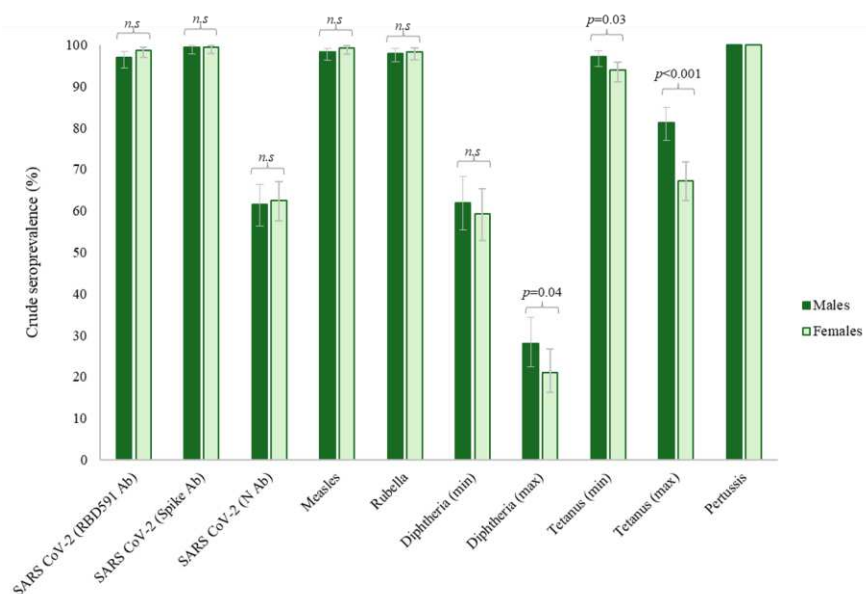
Pertussis Ab were detected in all participants (100%). The highest seroprevalence was for SARS CoV-2 *Spike* Ab (99.5%) and lowest for maximum protection against diphtheria Ab (33.1% [29.8-36.5%]). Compared to females, males had significantly higher seroprevalence for minimum protection against tetanus (97.2% vs 94.0%; $p=0.03$), maximum protection against tetanus (81.3% vs 67.3%; $p<0.001$), and maximum protection against diphtheria (28.0% vs 21.0%; $p=0.04$) (Figure 4, Panel A). When stratified by age group, seroprevalence was higher among <20-year-olds compared to other age groups for SARS CoV-2 *N* ($p<0.001$), maximum protection against tetanus ($p=0.01$), minimum protection against diphtheria ($p<0.001$), and maximum protection against diphtheria ($p<0.001$). Seroprevalence were highest among ≥ 50 -year-olds compared to younger age groups for measles ($p=0.01$) and were highest among 20-49-year-olds compared to other age groups for SARS CoV-2 *RBD591* ($p<0.001$) (Figure 4, Panel B). No significant associations were seen between VPDs and recruitment site (Figure 4, Panel C).

Significantly higher aOR for seropositivity were seen in participants aged ≥ 50 years and 20-49 years compared to participants aged <20 years for SARS CoV-19 *RBD591* Ab ($p=0.004$), with the opposite trend seen for SARS CoV-19 *N* Ab ($p<0.001$) (Supplementary Table 3c). Participants aged ≥ 50 years had significantly lower aORs for minimum protection against tetanus compared to <20-year-olds only (aOR=0.3 [0.1-0.9]; $p=0.03$), whilst 20-49-year-olds and ≥ 50 -year-olds had significantly lower odds of minimum protection (20-49-year-olds: aOR=0.2 [0.1-0.3]; $p<0.001$ and ≥ 50 -year-olds: aOR=0.1 [0.0-0.1]; $p<0.001$) and maximum protection (20-49-year-olds: aOR=0.3 [0.2-0.4]; $p<0.001$ and ≥ 50 -year-olds: aOR=0.1 [0.1-0.1]; $p<0.001$) against diphtheria.

A significant interaction was seen between age and sex for seropositivity for maximum protection against tetanus ($p=0.002$), with females aged ≥ 50 years having significantly lower odds of seropositivity (aOR=0.2 [0.1-0.5]; $p=0.01$) compared to females aged <20 years (Supplementary Table 3d).

283 SARS CoV-19 *RBD591* Ab predicted seropositivity was $\geq 95\%$ ($p < 0.001$) across all age groups in Niue.
 284 In contrast, predicted seropositivity for SARS CoV-19 *N* Ab was lower with increasing age from 71.7%
 285 in <20-year-olds to 53.8% in ≥ 50 -year-olds ($p < 0.001$). Predicted seropositivity for minimum protection
 286 against tetanus Ab were high across all age groups ($> 94\%$; $p < 0.001$) and decreased slightly with
 287 increasing age. Predicted seropositivity for minimum protection against diphtheria decreased significantly
 288 as age increased from 91.8% in <20-year-olds to 45.1% in ≥ 50 -year-olds. Similarly for maximum
 289 protection against diphtheria, predicted seropositivity decreased from 59.4% in <20-year-olds to 11.7% in
 290 ≥ 50 -year-olds. Predicted seropositivity for maximum protection against tetanus Ab increased with age
 291 among males (from 78.8% among <20-year-olds to 84.2% among ≥ 50 -year-olds; $p < 0.001$) and among
 292 females (from 85.2% in <20-year-olds to 52.1% in ≥ 50 -year-olds; $p < 0.001$) (Supplementary Table 4b and
 293 4c).

A)



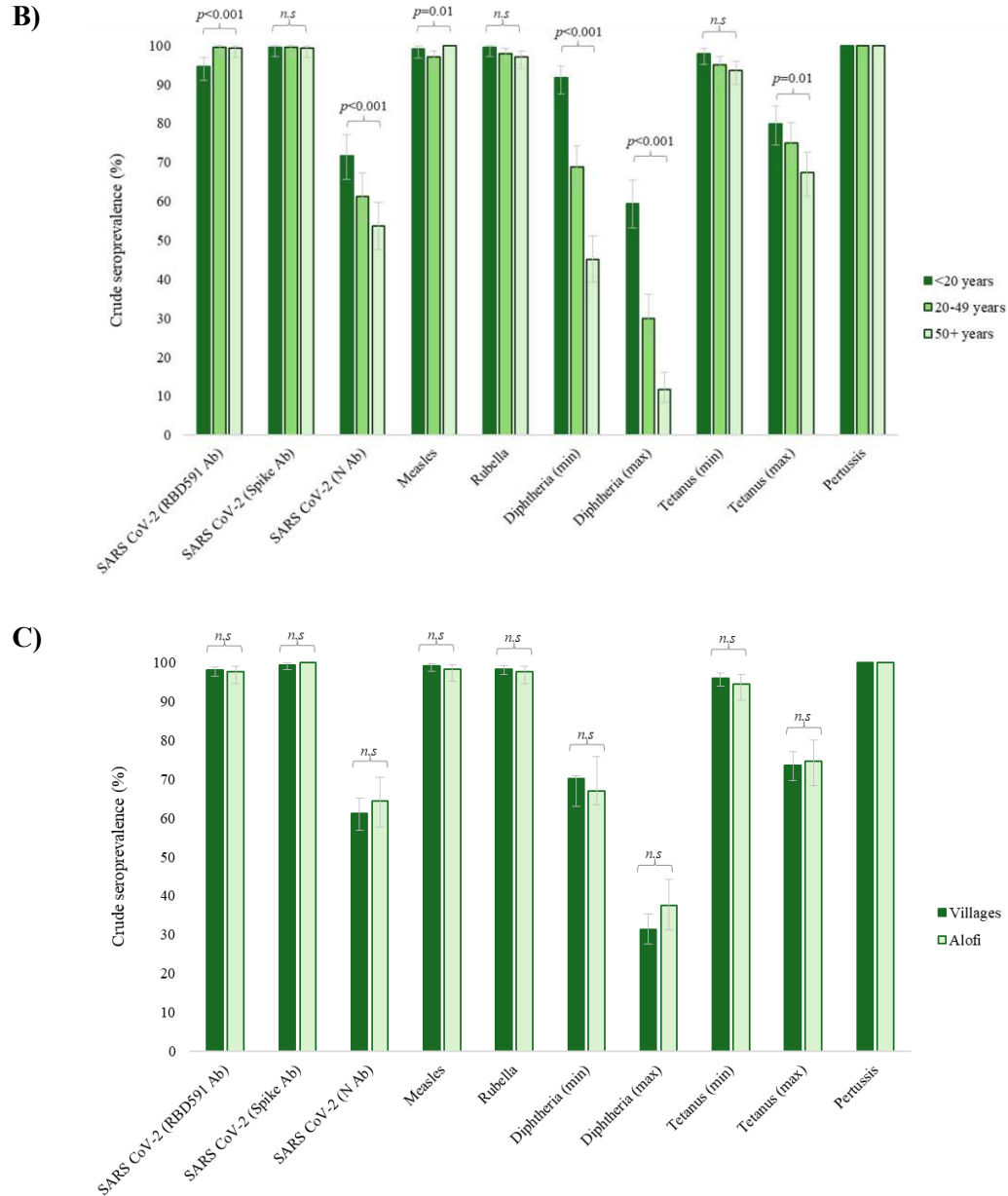


Figure 4: Seroprevalence of antibodies to vaccine preventable diseases in Niue in 2025 by sex (Panel A: males represented in dark green bars and females in light purple bars), age (Panel B: <20 years [dark green bar], 20-49 years [green bar], ≥50 years [light green bar]), and recruitment site (Panel C: Alofi represented by the light green bar and other villages represented by the dark green bar). Significance tested using univariable logistic regression. n.s = “non-significant”.

Seroprevalence to vaccine preventable diseases by 10-year age groups

Seroprevalence estimates for SARS CoV-2 RBD591 Ab was significantly between 10-year-age groups ($p=0.002$). Seroprevalence was lowest among 3-9-year-olds (84.3%) and was 100% for 20-29-year-olds,

40-49-year-olds, 50-59-year-olds, and ≥ 70 -year-olds. Similarly, seroprevalence for SARS CoV-2 *N* Ab was significantly different when stratified by 10-year-age groups ($p < 0.001$) and was lower, ranging from 40.4% among ≥ 70 -year-olds to 72.8% among 10-19-year-olds. For SARS CoV-2 *Spike* Ab, the lowest seroprevalence seen among 60-69-year-olds (98.2%) with 100% seropositivity for 10-19-year-olds, 20-29-year-olds, 40-49-year-olds, 50-59-year-olds, and ≥ 70 -year-olds (Table 2).

Significant differences in seroprevalence by 10-year age group was seen for maximum protection against tetanus ($p = 0.045$) and minimum ($p < 0.001$) and maximum ($p < 0.001$) protecting against diphtheria. Maximum protection for tetanus Ab ranged from 64.2% among 50-59-year-olds to 81.6% among 10-19-year-olds. For minimum protection against diphtheria, only 3-9-year-olds and 10-19-year-olds had a seroprevalence $\geq 90\%$ with the lowest seroprevalence among 60-69-year-olds (41.8%). Maximum protection against diphtheria did not exceed 61% for any age group and ranged from 60.9% among 10-19-year-olds to 10.1% among 50-59-year-olds. Seroprevalence estimates for measles Ab exceeded 95% for all age groups except 30-39-year-olds, of whom 93.9% were seropositive. Seroprevalence estimates for rubella Ab exceeded 95% for all age groups except in ≥ 70 -year-olds (93.6%). Seroprevalence estimates for minimum protection against tetanus were $< 95\%$ for participants aged 30-39 years (93.9%), 40-49 years (94.4%), 60-69 years (92.7%), and ≥ 70 years (91.5%) (Table 2).

320 **Table 2: Seroprevalence of antibodies to vaccine preventable diseases (VPDs), stratified by 10-year age groups, Niue 2025. P-value for age**
321 **group obtained using Wald χ^2 test (df=7).**

Disease (antigen)	Age group (years)																P-value		
	Total (n=750)		3-9 (n=70)		10-19 (n=174)		20-29 (n=69)		30-39 (n=82)		40-49 (n=89)		50-59 (n=109)		60-69 (n=110)			70+ (n=47)	
	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)		N	% (95% CI)
Seropositive for VPDs																			
Dengue (serotype DENV-1) DengNS1 sero1	200	26.7 23.6-30.0	0	0 -	9	5.2 2.7-9.7	16	23.2 14.7-34.6	28	34.2 24.7-45.0	34	38.2 28.7-48.7	46	42.4 33.3-51.7	51	46.4 37.3-55.7	16	34 22.0-48.6	<0.001
Dengue (serotype DENV-2) DengNS2 sero2	158	21.1 18.3-24.1	0	0 -	5	2.9 1.2-6.7	11	15.9 9.1-26.6	19	23.2 15.3-33.5	14	15.7 9.5-24.9	41	37.6 29.0-47.1	52	47.3 38.1-56.6	16	34 22.0-48.6	<0.001
Dengue (serotype DENV-3) DengNS3 sero3	162	21.6 18.8-24.7	0	0 -	6	3.5 1.6-7.5	11	15.9 9.1-26.6	19	23.2 15.3-33.5	15	16.9 10.4-26.1	42	38.5 29.9-48.0	53	48.2 39.0-57.5	16	34 22.0-48.6	<0.001
Dengue (serotype DENV-4) DengNS4 sero4	263	35.1 31.7-38.6	0	0 -	12	6.9 4.0-11.8	19	27.5 18.3-39.2	30	36.6 26.9-47.5	39	43.8 33.9-54.3	71	65.1 55.7-73.5	68	61.8 52.4-70.4	24	51.1 37.1-64.9	<0.001
Chikungunya (Chik E1)	24	3.2 2.2-4.7	0	0 -	7	4 1.9-8.2	3	4.4 1.4-12.7	7	8.5 4.1-16.9	4	4.5 1.7-11.4	0	0 -	3	2.7 0.9-8.1	0	0 -	0.45
Zika (Zika NS1)	23	3.1 2.0-4.6	0	0 -		2.3 0.9-6.0		10.1 4.9-19.8		6.1 2.6-13.8		4.5 1.7-11.4		0.9 0.1-6.2		1.8 0.5-7.0		0 -	0.04

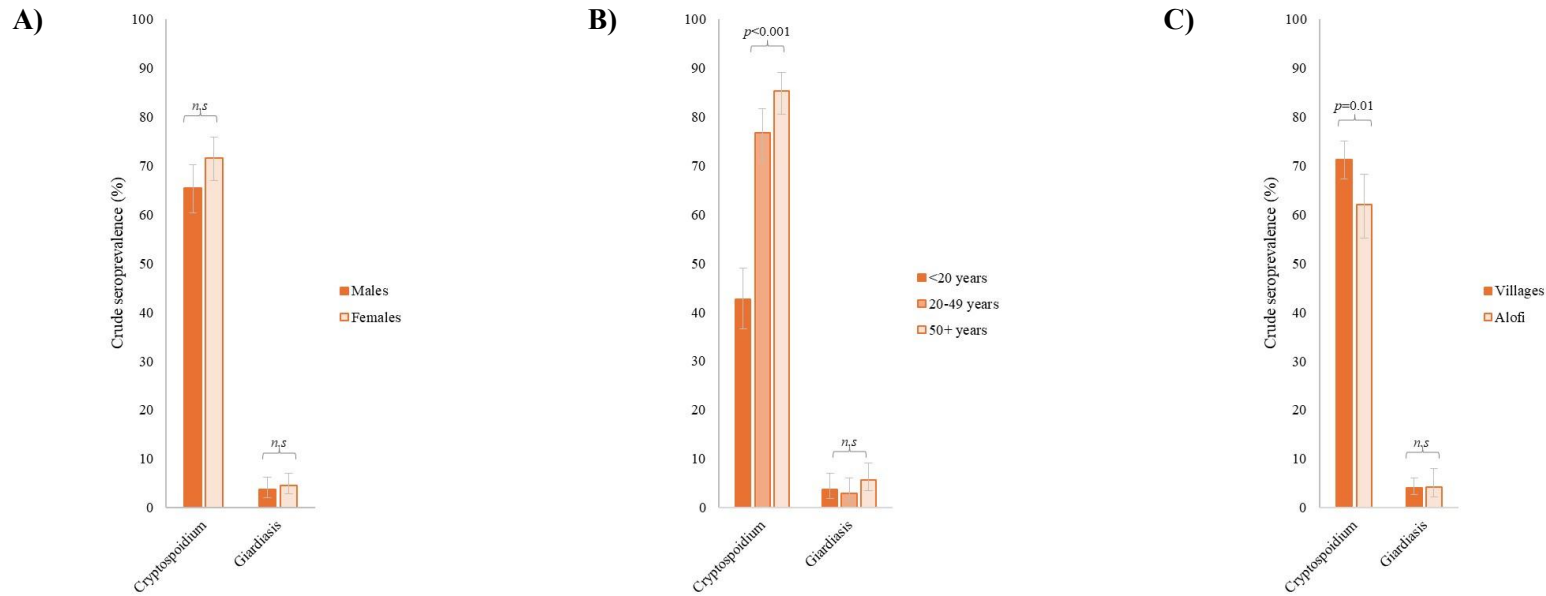
322
323

324 *Minimal threshold of protection: ≥ 0.01 IU/mL for tetanus toxoid and diphtheria toxoid; 0.153 IU/mL for measles; 9.36 IU/ml for rubella; 5 IU/mL*
325 *for pertussis. Maximum threshold of protection: ≥ 0.1 IU/mL for tetanus toxoid and diphtheria toxoid.*

Enteric protozoa

The overall seroprevalence of cryptosporidium was 68.7% (95% CI: 65.2-71.9); there was no significant difference by sex but seroprevalence was significantly higher in older age groups, ranging from 42.6% among <20-year-olds to 85.3% among ≥50-year-olds ($p<0.001$). Cryptosporidium seroprevalence was significantly higher among participants recruited from villages compared to Alofi (71.3% vs 62.0%; $p=0.01$). Giardiasis seroprevalence was much lower overall (4.1% [95% CI=2.9-5.8]), with no significant difference by sex, age group, or place of recruitment (Figure 6, Panel A-C).

Significantly higher aORs for seropositivity to cryptosporidium was seen among older age groups (20-49 years: aOR=4.4; $p<0.001$ and 50+ years: aOR=7.7; $p<0.001$) compared to <20-year-olds. Participants from Alofi had lower aOR for Ab for cryptosporidium compared with those recruited from other villages (aOR=0.7; $p=0.03$) (Supplementary Table 3e). Predicted seropositivity of cryptosporidium increased significantly with age among those recruited from other villages (from 46% among <20-year-olds to 87% among ≥50-year-olds; $p<0.001$) and from Alofi (from 36% among <20-year-olds to 81% among ≥50-year-olds; $p<0.001$) (Supplementary Table 4a).



341

342 **Figure 5: Seroprevalence of antibodies to enteric protozoa in Niue in 2025 by sex (Panel A: males represented in dark orange bars and**
 343 **females in light purple bars), age (Panel B: <20 years [dark orange bar], 20-49 years [orange bar], ≥50 years [light orange bar]), and**
 344 **recruitment site (Panel C: Alofi represented by the light orange bar and other villages represented by the dark orange bar). Significance tested**
 345 **using univariable logistic regression. n.s = “non-significant”.**

DISCUSSION

This study presents seroprevalence estimates of selected NTDs, arboviruses, VPDs, and enteric protozoa from a population-representative cross-sectional study embedded within a WHO STEPS survey conducted in Niue in 2025. Key findings were high seroprevalence for most VPDs, reflecting high reported vaccination coverage over the past two decades, and low seroprevalence for NTDs, suggesting low probability of ongoing transmission. Significant associations between age and arbovirus seroprevalence, and between age and sex with VPD seroprevalence, indicate potential opportunities for targeted arbovirus surveillance, prevention and control, and age-specific vaccination strategies in Niue.

In 2024, 99% of children under one year of age in Niue received age-appropriate doses of vaccines for measles, rubella, diphtheria, tetanus, and pertussis (DTP).[13] WHO recently announced that the block of 22 Pacific island countries and territories have been verified as having eliminated endemic circulation of measles and rubella.[21] However, the Pacific region has experienced severe measles outbreaks in recent years, most notably a devastating outbreak in Samoa in 2020 that resulted in 5,707 cases and 83 deaths (87% of which were in children aged <5 years) among children.[22] A cross-sectional study conducted in Samoa in 2019 prior to the outbreak found extremely low population-level measles Ab seroprevalence (43.6%),[2] coinciding with low reported vaccine coverage and highlighting the potential utility of MBA to identify vulnerable populations for targeted vaccination and outbreak prevention.

In contrast to Samoa, we found high seroprevalence of Ab against most VPDs in Niue which likely reflects high reported national vaccination coverage. Notably, seroprevalence for measles and rubella in Niue exceeded 95% (the commonly cited population immunity threshold required to sustain elimination) in all age groups, except 30-39-year-olds (93.9% for measles) and ≥ 70 -year-olds (93.6% for rubella). Despite high vaccination coverage, ongoing monitoring to guide vaccine strategies remains important, particularly in elimination settings where there is no circulating infection to boost immunity, and the risk of reintroduction is ever present.

Waning immunity has been proposed as one of the reasons for the reemergence of VPDs worldwide.[23] WHO recommends three doses of primary DTP-containing vaccine and two boosters at 12-23 months and 4-7 years for routine vaccine immunisation.[24] One tetanus-diphtheria toxoid booster dose is suggested for adolescents between 9-15 years of age.[24] A recent systematic review found evidence of waning immunity for diphtheria after vaccination and more long-term sustainable Ab against tetanus toxin.[24] Our estimates of minimum protection against tetanus and diphtheria (≥ 0.01 IU/mL) were lower than those for measles and rubella, particularly for diphtheria, and declined with increasing age, suggesting waning immunity in older age groups. For both diseases, the highest seroprevalence was seen among 10-19-year-olds, consistent with recommended tetanus-diphtheria booster doses in this age group. Seroprevalence estimates for maximum protection (≥ 0.1 IU/mL) for diphtheria and tetanus were 33.1% and 73.9% respectively, with similar decreases among older age groups. These findings highlight important immunity gaps, particularly in adults, and are consistent with recent diphtheria resurgence across the African Region, where outbreaks across at least eight countries have been linked to declining or insufficient population immunity.[25] In Niue, given the low protection (particularly for diphtheria), the DOH may wish to consider the introduction of booster doses for adults to reduce the risk of severe illness and outbreaks. Indeed, some countries recommend adult boosters every 10 years.[26]

High seroprevalence was seen to the SARS CoV-2 *RBD591* and *Spike* Abs, with comparatively lower seroprevalence for nucleocapsid (*N*) Abs. As of 31 December 2023, 100% of the Niuean population had received a complete primary series of a COVID-19 vaccine, with 76% having received at least one booster dose.[27] A total of 1,092 cases had been reported as of 29 March 2026. The observed pattern (higher seroprevalence of *RBD591* and *Spike* Ab alongside lower *N* Ab seroprevalence) could be considered consistent with a predominantly vaccine-derived immune profile, given that most COVID-19 vaccines induce *Spike*-specific Ab responses but not *N* Ab. Lower *N* Ab seroprevalence may reflect comparatively limited natural infection, although waning of *N* Ab over time and the timing of infection relative to vaccination may also contribute to this pattern. Nonetheless, these findings underscore the

395 importance of maintaining high vaccination coverage, including booster doses, to sustain population-level
396 protection.

397 Niue experiences occasional suspected dengue outbreaks and suspected circulation of chikungunya and
398 Zika. Dengue and other arboviruses have been circulating widely in other Pacific island countries and
399 territories in recent years.[28] Dengue transmission is strongly associated with population density,
400 movement, and urbanisation,[29, 30] thus given the geographical remoteness and limited accessibility of
401 Niue, it is not surprising that dengue seroprevalence was lower than that observed in other Pacific
402 settings.[2] Seroprevalence for dengue demonstrated clear cumulative exposure patterns with age,
403 reflecting historical and ongoing dengue transmission in the Pacific region. Interestingly, significantly
404 higher seroprevalence was seen in Alofi compared to other villages, suggesting spatial heterogeneity
405 consistent with higher urban transmission. Alternatively, higher dengue seroprevalence could reflect more
406 frequent travel to dengue-endemic countries by residents of Alofi, or a higher proportion of migrants to
407 Niue that reside in Alofi.

408 The use of serosurveillance to monitor pathogens that are not commonly part of routine surveillance, such
409 as NTDs, has been recognised as a valuable approach to improve disease surveillance and provide burden
410 estimates, particularly for low prevalence diseases that are approaching elimination, or that are considered
411 eliminated, as well as a means to monitor changes in seroprevalence over time.[31] Further, multi-
412 pathogen serosurveillance using platforms such as MBA, is increasingly recognised a cost-effective and
413 resource-efficient approach to monitor multiple diseases simultaneously.[7] Yaws and trachoma, two
414 NTDs targeted by WHO for eradication and elimination as a public health problem, respectably, are not
415 considered endemic in Niue, and hence the focus is on monitoring to detect resurgence or reintroduction
416 of these diseases. Reassuringly, zero participants were seropositive for yaws and only two participants
417 (aged 10 and 12 years) were seropositive for the trachoma *Pgp3* Ab. However, interpretation of *Pgp3* Ab
418 seropositivity should consider potential cross-reactivity, as *Pgp3* is expressed by both ocular *Chlamydia*
419 *trachomatis* (trachoma) and urogenital strains. Additional data, particularly in children aged 1-5

years,[32] would allow estimation of seroconversion rates and strengthen inferences about whether there is any trachoma transmission in Niue.

This study was designed as a post-validation surveillance activity to identify any persistent LF transmission following Niue's validation of elimination of LF as a public health problem.[14, 33] LF Ab seroprevalence estimates were low, reflecting LF Ag findings that have been discussed in detail elsewhere.[18]

To date, there is no specific data on the burden of *Strongyloides stercoralis* (strongyloidiasis) in Niue; however, a study conducted in Auckland involving Niuean migrants suggests strongyloidiasis may be endemic.[34] We found Ab seroprevalence of 1.9% for *Strongyloides*, with evidence of cumulative infection increasing with age. Similarly, there is no data on the endemicity status of *T. solium*, cryptosporidium or giardiasis. We found cysticercosis and taeniasis seroprevalence to be low overall (2.7% and 0.7%, respectively) but 70% of participants were seropositive for cryptosporidium Ab, a leading cause of diarrhoea morbidity and mortality globally in children <5 years.[35] We found only 4% of participants were seropositive for giardia Ab. Together, these findings warrant further investigation (and if required, intervention) to address potential environmental exposure pathways for these diseases; notably animal husbandry, water quality, and sanitation practices.

Despite important progress in reducing several communicable diseases, including those targeted for elimination, Pacific island countries and territories remain vulnerable to disease outbreaks, re-emerging diseases, and persistent transmission driven by climate change and extreme weather events, other environmental change, challenges in reaching remote communities, and fragmented financing.[36, 37] These pressures underscore the importance of robust surveillance to support national health priorities, including safeguarding disease elimination gains. Integrating serosurveillance within the WHO STEPS survey platform enabled efficient use of existing survey infrastructure, logistics, field teams, and community engagement processes, reducing duplication and potentially improving cost-efficiency

compared with stand-alone infectious disease surveys. This approach also allowed simultaneous collection of data on multiple communicable and non-communicable health priorities from a single survey platform, which is particularly valuable in small island settings with constrained workforce and financial resources. However, integration also increased operational complexity, including additional training requirements, laboratory coordination, specimen management, and cross-programmatic collaboration to ensure both NCD and infectious disease objectives were achieved. Nonetheless, our findings demonstrate that embedding integrated serosurveillance within existing national survey platforms is feasible and may provide a pragmatic and scalable model for strengthening surveillance in Pacific Island countries and other resource-constrained settings.

A limitation of this study, and of serosurveys more broadly, is the need for continued work to strengthen interpretation of Ab responses, including standardisation of assays to international units, improved understanding of inter-laboratory variability, refinement of seropositivity cut-offs, and careful consideration of cross-reactivity between related pathogens. Furthermore, cross-reactivity between arbovirus serotypes limits the ability to distinguish circulating dengue serotypes and other arboviruses serologically; therefore, differences in seropositivity between serotypes should be interpreted with caution. Collectively, our findings demonstrate the potential of integrated multi-pathogen serosurveillance for characterising transmission dynamics and informing targeted surveillance and intervention strategies in Niue. We have provided a snapshot of seroprevalence across 17 pathogens in Niue, establishing a baseline for longitudinal monitoring and evaluation. Importantly, this study adds to the growing evidence supporting MBA as a feasible tool for national and regional surveillance systems.

CONCLUSION

Overall, our findings demonstrate the utility of integrating multi-pathogen serosurveillance within a WHO STEPS survey, with MBA platforms providing an efficient approach to generate high-resolution surveillance data to support more informed and integrated public health strategies, including approaches

468 to sustain and achieve VPD and NTD elimination goals.[38] If multiple serosurveys are conducted over
469 time, results can be used to track the impact of public health interventions, or the impact of climate and
470 environmental change on disease transmission. Importantly, if deployed through existing survey
471 platforms and data pipelines, MBA can strengthen early detection of under-vaccination, emerging
472 infections, and persistent transmission, thereby enhancing the global capacity to achieve and sustain
473 elimination of NTD and VPDs.[31]

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AUTHOR CONTRIBUTIONS

Conceptualisation: ATC, HLSL GM, and CLL. Investigation: HLSL, ATC, GM, PT, MN, AM, TM, LJR, and FA. Data curation: HLSL, ATC. Project administration: ATC. Analysis: HLSL, ATC, LJR, and FA. Supervision: CLL. Writing - original draft: HLSL. Writing - review and editing: all authors. All authors had access to the raw data. All authors reviewed the final manuscript. HLSL had responsibility for the decision to submit for publication.

DATA AVAILABILITY

Communities in Niue are small (some with fewer than 10 inhabitants), and sharing individual-level data could enable the identification of individual participants, violating the conditions of the study's ethics approval. For requests related to data access, please contact the Niue Department of Health Human Ethics Committee via the Niue Public Health Unit's focal point (grizelda.mokoia@gov.nu).

DECLARATION OF INTERESTS

We declare no competing interests.

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