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Integrated multi-pathogen serosurveillance in children from East New Britain, Papua New Guinea using multiplex bead assays

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ABSTRACT

Children in Papua New Guinea experience a high burden of infectious diseases, including neglected tropical diseases, arboviral infections, enteric pathogens, and soil-transmitted helminths. Multipathogen serological surveillance provides a powerful approach for simultaneously characterising population-level exposure and susceptibility to multiple pathogens, complementing other forms of surveillance and to guide public health decision-making. We collected blood samples from 379 children aged 10 to 24 months (median 16 months) living in East New Britain Papua New Guinea, to assess antibodies to 11 priority pathogens. Seropositivity was highest for *Cryptosporidium* (52.8%), followed by dengue (30.9%), chikungunya (25.3%), *Giardia* (13.5%) and malaria parasites (*Plasmodium vivax* 11.1%; *Plasmodium falciparum* 5.0%). Males were significantly more likely to be positive for trachoma (10.5% vs 2.2%, $p = 0.001$). Co-exposure to multiple pathogen groups was common, with approximately 45% of children seropositive for antibodies from two or more different pathogens. While prevalence varied markedly across pathogen groups, these results confirm substantial early-life exposure to multiple endemic pathogens in East New Britain, Papua New Guinea. Simultaneous assessment of multiple pathogens from a single sample demonstrates the value of multiplex serological surveillance and can inform targeted public health interventions.

INTRODUCTION

Serological surveillance provides a powerful approach for characterising population-level exposure and susceptibility to infectious diseases, offering insights that complement routine case-based and genomic surveillance^[1]. By measuring pathogen-specific antibodies, serosurveys capture cumulative exposure and immune responses that are often missed by conventional surveillance systems, particularly in low- and middle-income settings. In Papua New Guinea (PNG), geographic, logistical, and health system constraints limit the availability and representativeness of routine surveillance data, underscoring the need for scalable approaches that can generate robust epidemiological evidence from existing studies and specimens^[2].

Children in PNG experience a high burden of infectious diseases, including neglected tropical diseases, arboviral infections, enteric pathogens, and soil-transmitted helminths, often with repeated or

concurrent exposures beginning early in life ^[3-6]. These infections are frequently asymptomatic or underdiagnosed yet can have substantial cumulative impacts on child health. Chronic or recurrent enteric infections and soil transmitted helminths have been associated with impaired linear growth, undernutrition, anaemia, and altered immune function, while repeated arboviral and other endemic infections may further contribute to inflammatory and immunological stress during critical periods of development ^[7,8]. Understanding patterns of exposure to multiple pathogens in childhood is therefore essential for informing integrated disease control strategies and broader child health interventions.

Multiplex bead assay (MBA) technologies enable the simultaneous measurement of antibodies to antigens from multiple pathogens using a single, small-volume specimen. Integrating MBA-based multi-pathogen serosurveillance into demographic health or disease-specific surveys allows efficient assessment of exposure to vaccine preventable diseases, neglected tropical diseases, arboviruses, enteric pathogens, and soil transmitted helminths within the same cohort ^[9]. This integrated approach supports the identification of co-exposure patterns and overlapping disease burdens that would be difficult to detect using pathogen-specific studies alone. As validated antigen targets continue to expand, MBA platforms also facilitate cross-pathogen analytical approaches that can inform prioritisation of interventions and contribute to efforts to reduce health inequities among children in PNG.

This study is a serological sub study nested within a larger study designed to assess population-level immunity to vaccine-preventable diseases in young children in PNG. Leveraging stored samples from the parent study provides a unique opportunity to extend the scientific value of existing data by characterising exposure to a broader range of pathogens without additional sample collection or participant burden ^[10]. Such an approach is particularly advantageous in paediatric populations, where blood volumes are limited and repeated sampling is ethically and logistically challenging.

In this serological sub study, we investigated antibody seroprevalence to 20 antigens from 11 pathogens, including neglected tropical diseases, soil transmitted helminths, malaria, arboviruses, and enteric pathogens, among children aged 10 to 24 months residing in East New Britain Province (ENB), PNG. Children were recruited from five priority local-level government (LLGs) areas encompassing urban, rural, and remote island settings, selected to capture geographic and sociodemographic diversity across

ENB. This integrated, multi-pathogen approach provides a comprehensive assessment of early-life exposure to endemic infections and generates evidence to inform coordinated child health and disease control strategies in the province.

RESULTS

Demographic characteristics of the study participants is summarised in Table 1. A total of 379 children aged 10-24 months, with a median age 16 months were enrolled between late March and mid-August 2023. Participant numbers were broadly comparable across the five targeted LLG areas, and just over half of the cohort were male (53%).

Table 1: Demographic characteristics of participants

	N (%)
Gender	
Male	200 (53%)
Female	179 (47%)
Age (months)	
<12	70 (18%)
12 – <18	169 (45%)
18 – <24	136 (36%)
<i>Missing</i>	4 (1%)
Local-level government	
Bitapaka	78 (21%)
Duke of York	77 (20%)
Inland Baining	66 (17%)
Kokopo Urban	81 (21%)
Toma-Vunadidir	77 (20%)

Seroprevalence of pathogens in children

Among the 379 children, antibodies to multiple pathogens were detected, with substantial variation in prevalence across the 11 pathogen groups included in the study (Supplementary Figure 1). Seropositivity was highest for *Cryptosporidium* (*Cryptosporidium parvum*) (52.8%), followed by dengue virus (30.9%), chikungunya virus (25.3%), *Giardia* (*Giardia lamblia*) (13.5%), *Plasmodium vivax* (11.1%), trachoma (*Chlamydia trachomatis*) (6.6%), *Plasmodium falciparum* (5.0%), soil-transmitted helminths (*Strongyloides stercoralis* or *Taenia solium*) (2.9%), lymphatic filariasis (1.9%), Zika virus (1.6%) and yaws (*Treponema pallidum*) (0%).

No significant differences in seroprevalence were observed between males and females for most pathogens. However, males were significantly more likely (10.5% vs 2.2%, $p = 0.001$) to have been exposed to *C. Trachomatis* (Pgp3). Co-exposure to multiple pathogen groups was common. Of the 11 pathogens examined, approximately 25% (N = 93) of children were seropositive for antibodies from two different pathogens, and an additional 20% (N = 77) were seropositive to three or more pathogens, confirming significant exposure to malaria, arboviruses, and neglected tropical diseases in early childhood.

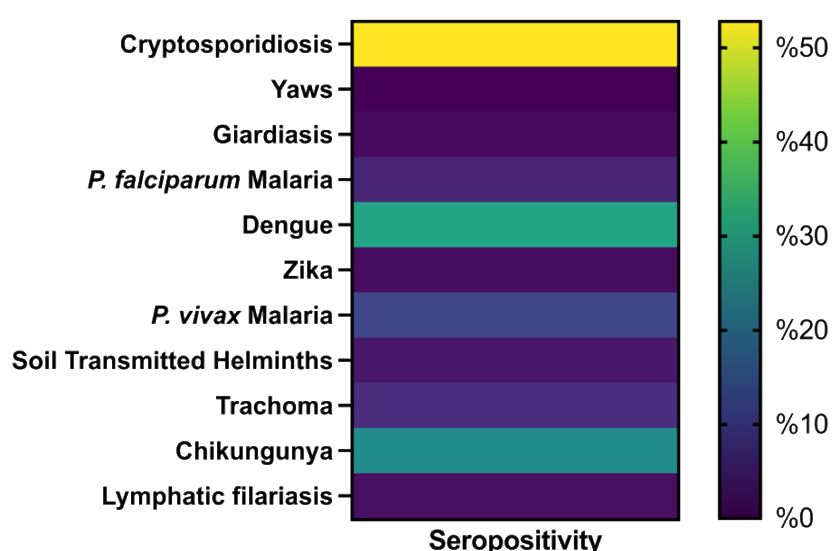


Figure 1. Heatmap showing overall seroprevalence for the 11 pathogens examined in the study (N = 379).

Does exposure accumulate with age?

Serological responses demonstrated substantial heterogeneity in exposure across pathogens and age groups. Antibody responses to *Cryptosporidium* were consistently high across all age strata, suggesting that exposure occurs early in life. Similarly, seroreactivity to *P. vivax* was common across the study population, indicating sustained transmission.

Clear age-related trends were observed for several pathogens. Seropositivity increased significantly with age for yaws ($p = 0.014$), dengue virus ($p < 0.001$), and chikungunya virus ($p < 0.001$), indicative of increased likelihood of environmental and vector exposure with age. (Figure 2).

For several infections, including trachoma ($p = 0.035$), *P. falciparum*, *Giardia*, and soil-transmitted helminths, relatively high seroprevalence was observed among children younger than 12 months and may reflect a combination of maternally derived antibodies and natural exposure. Consistent with this, we observed a rise in seropositivity to *P. falciparum*, trachoma, *Giardia* in children aged >18 months, but not 12-18 months, suggesting that antibody responses later in life reflect true exposure and infection acquired during early childhood.

Pathogen-specific differences were also evident among the youngest infants. No serological evidence of Zika virus exposure was detected in children younger than 12 months, while detectable antibodies were observed in a small proportion of those children older than 12 months (1.6%). This finding suggests either an absence of recent transmission or very limited exposure within the study population, particularly among younger age groups.

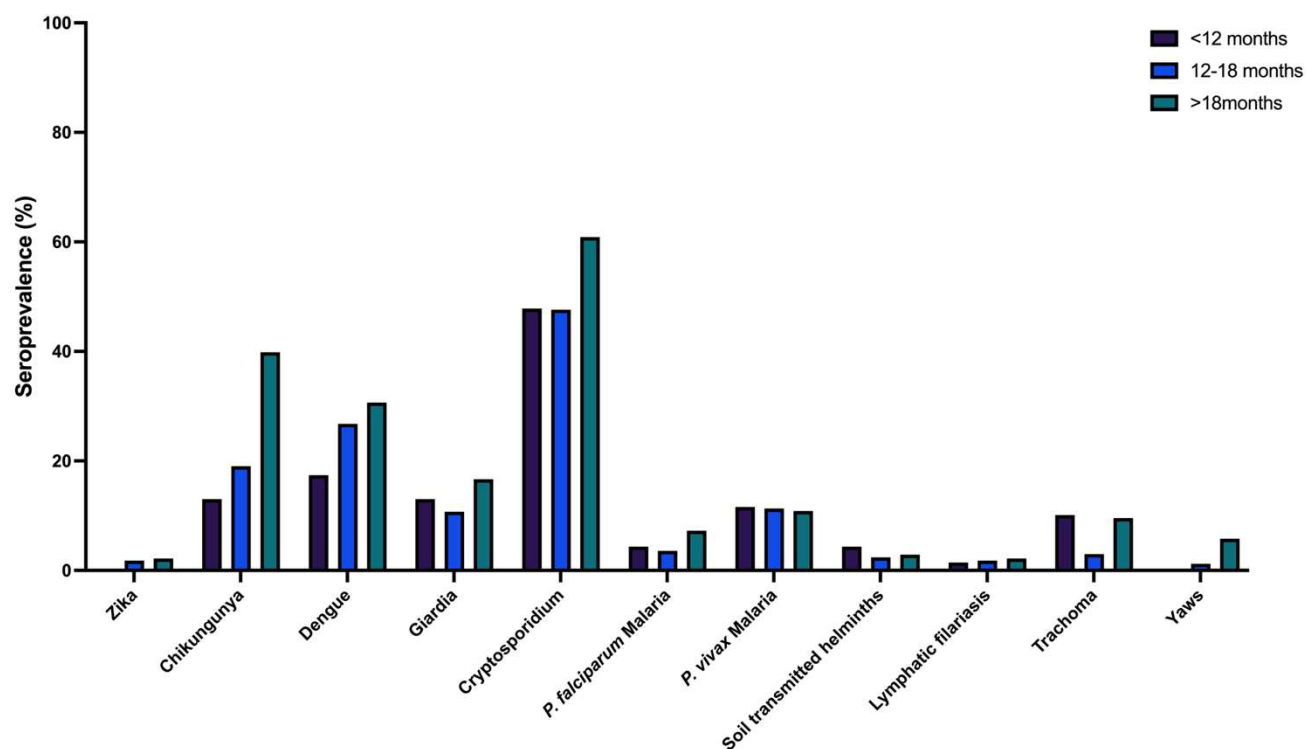


Figure 2. Seroprevalence by age group (N = 379).

Where is transmission concentrated?

Seroprevalence varied across the five local-level government areas despite relatively similar numbers of samples collected from each location, reflecting underlying geographic and sociodemographic heterogeneity across the study sites, which included urban, rural, and remote island settings (Figure 3). These spatial differences suggest heterogeneous patterns of pathogen exposure within the study province.

Antibodies to *P. vivax* malaria were most prevalent in the Duke of York and Toma-Vunadidir LLGs, indicating higher levels of exposure in these areas compared with the other study sites. In contrast, serological evidence of trachoma (based on responses to *C. trachomatis* antigens) was markedly lower in the Duke of York LLG. Arboviral exposure also showed geographic variation, with antibodies to chikungunya most frequently detected among children living in the Bitapaka and Kokopo Urban LLGs.

Marked differences were also observed for several other pathogens. Notably, no serological evidence of exposure to *P. falciparum* malaria or lymphatic filariasis was detected among children sampled from the Inland Baining LLG. In contrast, almost half of children with antibodies to *Giardia* were from Toma-Vunadidir, suggesting higher levels of transmission of this enteric pathogen in that area. Together, these findings highlight substantial spatial heterogeneity in exposure to malaria, arboviruses, enteric pathogens, and neglected tropical diseases within the study region.

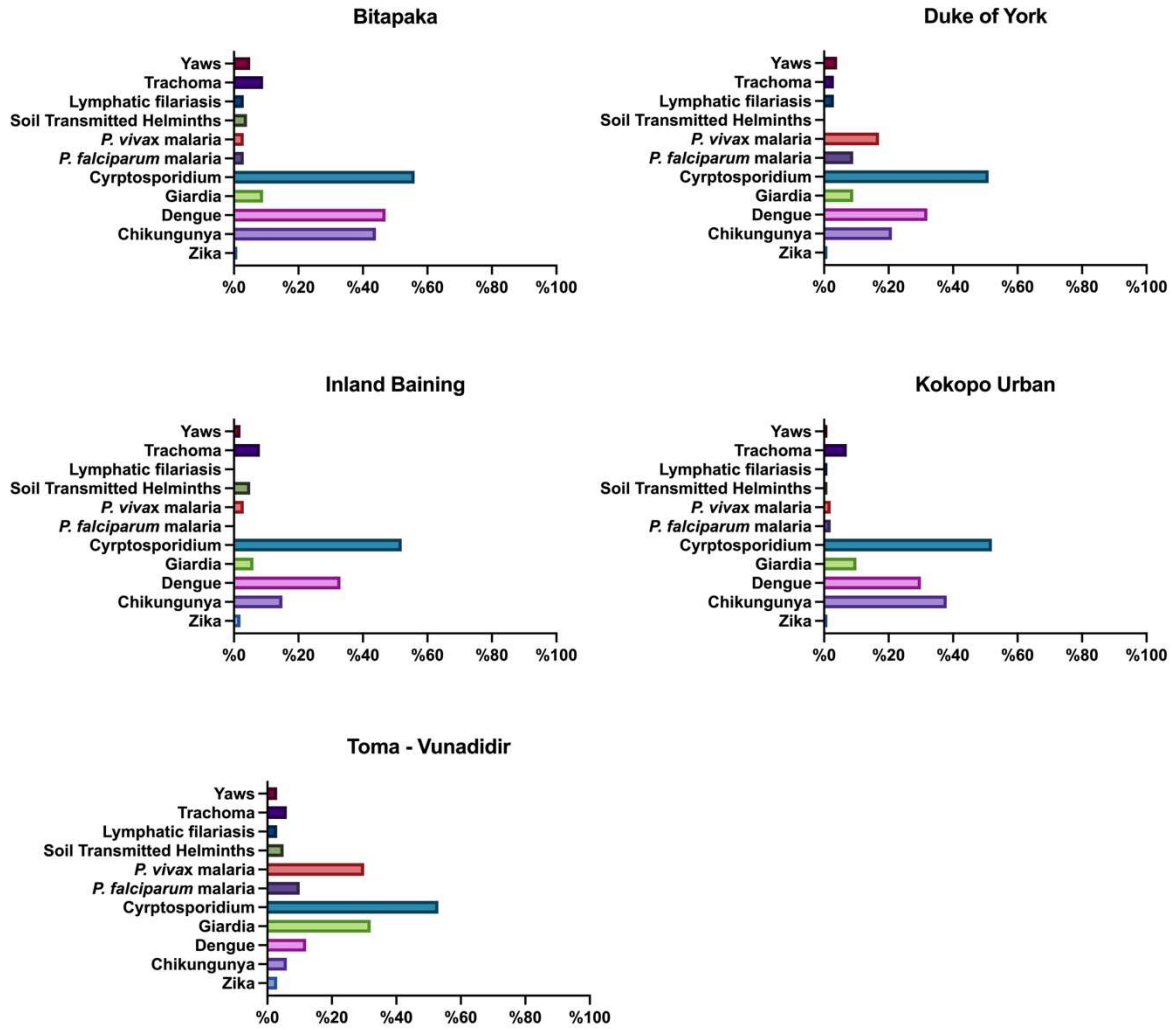


Figure 3. Geographic variation in seroprevalence across five local-level governments (N = 379).

DISCUSSION

To our knowledge, this is the first study to apply integrated multi-pathogen serosurveillance in children in PNG, demonstrating the feasibility and added value of this approach for characterising early life exposure to multiple co-endemic pathogens in this setting. Overall, 45% of children had detectable antibodies to multiple pathogens, although pathogen-specific seroprevalence varied by age and geographic location. Seropositivity was highest for *Cryptosporidium*, followed by the arboviruses dengue and chikungunya, then *Giardia*, trachoma, and malaria parasites *P. vivax* malaria and *P. falciparum* malaria. Seroprevalence for dengue was similar to that of chikungunya, reflecting comparable early-life exposure in this population. The high prevalence of antibodies to enteric pathogens such as *Cryptosporidium* and *Giardia* suggests that exposure occurs very early in life, likely reflecting ongoing transmission associated with limited access to clean water, sanitation, and hygiene conditions. While it is widely acknowledged that malaria, particularly *Plasmodium vivax*, is commonly experienced by children in this age group in PNG ^[11,12], these findings provide serological evidence that exposure to dengue and chikungunya is similarly prevalent. Data from Pacific Island serosurveys support substantial dengue exposure occurring in early childhood, with seroprevalence among children under five years already approaching 40–50% in some settings ^[13,14]. This is important for informing both clinical management of febrile illness in young children and the design of targeted public health interventions.

Overall, antibodies to enteric pathogens and malaria were most commonly detected, while neglected tropical diseases such as soil-transmitted helminths, trachoma, yaws, and lymphatic filariasis were generally less prevalent. Patterns of seropositivity varied across antigens, suggesting heterogeneous transmission dynamics within the province and highlighting the value of multi-pathogen serological surveillance in this young paediatric population. In 2022, East New Britain Province completed two annual rounds of lymphatic filariasis mass drug administration with the triple drug regimen ivermectin, diethylcarbamazine, and albendazole (IDA) ^[15]. The limited serological evidence of exposure observed in this study of children surveyed in 2023 is consistent with reduced or interrupted transmission in two LLGs ^[16]. The presence of detectable antibodies in a subset of children in 3 LLGs suggests that

transmission likely persists within some areas of ENB ^[16]. Seroprevalence was generally similar between males and females, suggesting comparable exposure risks during early childhood. The exception was the Pgp3 antigen of *C. trachomatis*, for which seropositivity was significantly higher in males. This has not been previously observed in other studies of Pgp3 IgG seroprevalence to *C. trachomatis* in children, which have identified age and community transmission intensity as they key drivers of exposure ^[17]. Conversely, adult women have been reported to have substantially higher rates of trichomatous scarring and trichiasis than men, widely attributed to gendered exposure over a lifetime ^[18].

Evidence of exposure to multiple pathogens was common. Approximately one-quarter of children were seropositive for antibodies to two pathogens, and a further one-fifth showed evidence of exposure to three or more pathogens. This pattern highlights the considerable infectious disease burden faced by young children in this setting, where malaria parasites, arboviruses, enteric pathogens, and neglected tropical diseases circulate concurrently. The age-stratified analyses further support this interpretation. Seropositivity increased with age for several pathogens, including dengue, chikungunya, lymphatic filariasis, and yaws, consistent with increasing interaction with people, vectors or environment as children get older. In contrast, seroreactivity to *Cryptosporidium* remained high across age groups, suggesting that exposure occurs very early in life. Reduced antibody responses among children aged 12–28 months for several pathogens, including trachoma, *P. falciparum*, *Giardia*, and soil-transmitted helminths, may reflect a combination of limited early exposure, waning maternal antibodies, or variability in transmission intensity ^[19,20]. Maternal antibodies typically wane between 3–9 months after birth and therefore are unlikely to fully explain seroreactivity observed later in infancy ^[11]. The absence of detectable antibodies to Zika virus in children younger than 12 months further suggests limited recent transmission within this cohort. Overall, these findings indicate that exposure accumulates with age for several pathogens, particularly vector-borne and neglected tropical diseases, while other infections show evidence of early-life exposure or maternally derived immunity. The contrasting patterns likely reflect differences in transmission routes, environmental exposure, and infection dynamics across pathogens within the study setting.

Clear geographic differences in seroprevalence were also observed across the five LLG areas sampled. Higher seroprevalence of *P. vivax* in Duke of York and Toma-Vunadidir suggests greater malaria exposure in these areas, potentially reflecting ecological or environmental factors that influence mosquito vector abundance. In contrast, the absence of detectable antibodies to *P. falciparum* and lymphatic filariasis in the Inland Baining area may indicate lower transmission intensity or limited recent exposure. Distinct spatial patterns were also evident for other pathogens, including higher seroprevalence of chikungunya and dengue in Bitakpa and Kokopo Urban LLG areas, and increased *Giardia* exposure in Toma-Vunadidir. These findings highlight substantial spatial heterogeneity in pathogen transmission within ENB province, likely influenced by differences in environment, population density, water and sanitation infrastructure, and vector ecology.

This study has several limitations that should be considered when interpreting the findings. First, the cross-sectional design limits the ability to infer the timing of infection or changes in transmission over time, as antibody responses could reflect cumulative exposure or recent infection. Second, while multiplex serology provides a sensitive method for detecting pathogen exposure, antibody responses can persist for varying durations depending on the pathogen and antigen, which may influence estimates of seroprevalence. Third, the study was limited to young children and may not capture exposure patterns in older age groups, where cumulative exposure is likely to be higher. Additionally, although sampling was distributed across multiple LLG areas, the sample size within each location may limit the precision of subnational comparisons. Finally, serological assays may be subject to cross-reactivity between related pathogens, particularly among arboviruses ^[21-23], which could influence interpretation of exposure patterns.

Despite these limitations, this study demonstrates the utility of multiplex serological approaches for assessing exposure to a wide range of pathogens within population-based surveys. In settings such as PNG, where multiple infectious diseases co-circulate and surveillance resources are often limited, integrated multi-pathogen serosurveillance provides an efficient means of monitoring transmission dynamics and identifying overlapping disease risks. The ability to assess exposure to malaria,

arboviruses, enteric pathogens, and neglected tropical diseases from a single sample enables more comprehensive insights into population-level infection patterns.

Taken together, these findings indicate that children in this setting are exposed to a diverse array of pathogens from an early age, with transmission dynamics varying by pathogen, age group, and geographic location. Multiplex serological platforms therefore offer a valuable tool for strengthening integrated surveillance systems, supporting more coordinated public health strategies, and informing targeted interventions in complex endemic settings.

METHODS

Ethics statement

The protocol was approved by the Alfred Health Ethics Committee, Australia (#202/22), the PNG Institute of Medical Research Institutional Review Board (#2206) and the PNG Medical Research Advisory Committee (#22.52). Written informed consent was obtained from parents or caregivers prior to undertaking any study procedures. All experiments were performed in accordance with the relevant guidelines and regulations.

Dried Blood Spot collection

Fingerpick or heel prick blood was collected onto filter paper, dried and placed in sealed plastic bags with desiccant. Dried blood spots (DBS) were transported to Papua New Guinea Institute of Medical Research in Port Moresby and placed in cold storage at -20°C.

Multiplexed Bead Assay

IgG antibodies against 3 neglected tropical diseases (lymphatic filariasis, yaws and trachoma), 3 soil transmitted helminths (taeniasis, strongyloidiasis, neurocysticercosis), 2 malaria species (*P. falciparum* and *P. vivax*) 3 arboviruses (Zika, chikungunya and dengue serotypes 1-4) and 2 enteric pathogens (*Giardia* and *Cryptosporidium*) were measured using a magnetic bead-based Luminex multiplex assay. Dried blood spots (6 mm diameter, ~10 µL whole blood) were eluted overnight at 4 °C in PBS containing 0.5% casein, 0.3% Tween 20, 0.5% polyvinyl alcohol, 0.8% polyvinylpyrrolidone, 0.02%

sodium azide, and 3 µg/mL E. coli extract (Buffer B). Eluates were diluted 1:400 in Buffer B prior to assay.

For each antigen, 1250 antigen-coupled beads per well were incubated with 50 µL of diluted sample for 1.5 h. Wells were washed three times with PBST (0.3% Tween 20) and incubated with 50 ng biotinylated mouse anti-human IgG and 20 ng biotinylated mouse anti-human IgG4 (Southern Biotech, Birmingham, AL) for 45 min. After three washes with PBST, 250 ng phycoerythrin-labelled streptavidin (Invitrogen, South San Francisco, CA) was added for 30 min. Beads were washed again and incubated with 50 µL PBS containing 0.5% BSA, 0.05% Tween-20, and 0.02% sodium azide to remove loosely bound antibodies, followed by a final wash. Beads were resuspended in 100 µL PBS and stored overnight at 4 °C. For further information on bead coupling please contact corresponding author.

Plates were read the next day on a MAGPIX instrument (Luminex, Austin, TX). Median fluorescence intensity (MFI) values were recorded for each antigen, and background (bg) fluorescence from blank wells (Buffer B only) was subtracted to obtain MFI-bg^[24].

Determination of the threshold for antigen seropositivity

Different cut-off methods were used to determine seropositivity per antigen (Supplementary Table 1). The mean plus three standard deviations of 193 samples from citizens of the USA with no history of foreign travel were used to estimate cutoffs to 10 antigens on this panel. Known positive and negative samples were used to determine seropositivity cut-offs to 10 antigens on this panel. Cutoffs were determined using ROC analysis as previously described (Supplementary Table 1)^[25]. For further information on cutoff methods please contact corresponding author.

Determination of pathogen seropositivity

Seropositivity at the pathogen level was determined based on antigen-specific responses measured in the multiplex assay. For most pathogens, a sample was classified as seropositive if it exceeded the predefined seropositivity threshold for any antigen within the corresponding pathogen antigen panel. This approach was used to maximise sensitivity for detecting prior exposure, recognising that antibody responses may vary between individuals and across antigens within the same pathogen.

An exception was made for yaws (*Treponema pallidum* subsp. *pertenue*). For this pathogen, samples were classified as seropositive only if they were positive for both antigens included in the assay. This more stringent criterion was applied due to documented cross-reactivity between one TmpA antigen and antibodies generated against *Treponema pallidum* subsp. *pallidum* (syphilis). Requiring dual-antigen positivity therefore improved specificity and reduced the likelihood of misclassification due to cross-reactive antibody responses (Supplementary Table 1).

Statistical analysis

Statistical analyses were performed using Stata v18.0 (StataCorp, TX, USA). Demographic characteristics, including age and gender, were summarised using frequency counts and percentages. Comparisons of antigen positivity between groups (e.g., by LLG) using chi-squared tests, with significance defined as $p < 0.05$.

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Author Contributions

Conceptualisation and funding acquisition: MOK, SV, LJR, FA, PK, EW, ML and MD. Methodology: FA, LJR. Data collection: SV, PM, MD and DBS collectors. Supervision: MOK and LJR. Formal analysis: SV, FA, MOK and LJR. Antibody assays: DM, DR and FA. Data interpretation: MOK, SV and FA, LJR. Writing (original): SV and FA. MOK, SV, LJR and FA verified the data in the study and take full responsibility for the integrity of the data. All authors have read and approved the final manuscript.

Additional information

The authors declare no competing interests.

Data availability statement

All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

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Figure legends

Figure 1. Heatmap showing overall seroprevalence for the 11 pathogens examined in the study (N = 379). Colour intensity represents the proportion of samples classified as seropositive for each pathogen based on antigen-specific thresholds derived from the multiplex bead assay. For most pathogens, samples were considered seropositive if antibody responses exceeded the threshold for at least one antigen within the pathogen-specific panel. For yaws (*Treponema pallidum* subsp. *pertenue*), samples were classified as seropositive only if responses exceeded the threshold for both antigens to minimise misclassification due to cross-reactivity with syphilis (*Treponema pallidum* subsp. *pallidum*). Seroprevalence is presented as the percentage of seropositive individuals among the total samples tested.

Figure 2. Seroprevalence by age group (N = 379). Bars show the proportion of individuals within each age category who were classified as seropositive for each pathogen. Age-specific trends highlight differences in exposure or immunity across the population.

Figure 3. Geographic variation in seroprevalence across five local-level governments (N = 379). Bars represent the proportion of seropositive individuals in each LLG, highlighting spatial differences in exposure to the pathogens examined.

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