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Long-term dynamics of cytomegalovirus-specific antibodies in a longitudinal cohort of children followed up for the first decade of life

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Cytomegalovirus (CMV) causes infections that last a lifetime and are primarily contracted in childhood. Congenital transmitted CMV is associated with severe neurological sequelae and can cause life-threatening disease in immunocompromised individuals. Although antibodies are generally presumed to correlate with protection, their long-term dynamics remain poorly understood. We aimed to determine the longevity of CMV-specific antibodies in a low-income setting in Kilifi County, Kenya. To track long-term antibody dynamics, we conducted longitudinal surveillance of annually collected serum samples and assayed for antibodies against the CMV tegument phosphoprotein (pp150). The duration of effective immunity against re-infection was estimated using piecewise regression modelling. Serum antibody to CMV was measured in 123 children recruited within the first five years of life and sampled annually over a median of 10 years (range: 7–14). Antibodies to the CMV pp150 showed a cyclic trend of acquisition and loss at the population level. Individually, we observed early antibody acquisition, followed by decline and rebound. Regression analysis identified a 7.57-year inflection point in antibody trajectories, marking a transition from waning to re-accumulation - potentially reflecting natural boosting events in a population where CMV infection occurs early in life. The data show a clear pattern of early natural infection, followed by repeated patterns of antibody acquisition, loss, and re-acquisition over the first decade and a half of life.

Keywords Cytomegalovirus (CMV), Antibody kinetics, Longitudinal analysis, Sub-Saharan africa, Children

Cytomegalovirus (CMV) is a DNA virus and belongs to the Herpesviridae family and beta herpesvirus subfamily[1]. The CMV seroprevalence among the adult population in most developing countries is about 70%, while in developed countries, it is about 60%[2–4]. Most CMV infections are typically asymptomatic and lifelong and establish latency in myeloid cells[5–7]. This ability to establish latency and reactivate makes CMV a significant pathogen, particularly in immunocompromised individuals and pregnant women[8]. In children, congenital CMV is the leading cause of neuro-sensory hearing loss, cognitive impairment, and cerebral palsy at birth[5,9]. Maternal CMV antibodies circulate up to 12 months after birth; thereafter, the presence of the antibodies indicates a primary infection[10]. Currently, there are no licensed vaccines globally, however, clinical trials for the vaccine candidates are ongoing[11–13].

There is a paucity of data on the long-term dynamics of CMV-specific antibodies from childhood to early adulthood. Previous seroprevalence studies have predominantly been cross-sectional or limited to single time point sampling[14–16]. The differential age prevalence of CMV antibodies in high and low-income settings, suggests underlying differences in epidemiology and transmission and highlights the need to track the patterns of antibody acquisition and maintenance in the first few years of life. Understanding these kinetics is important

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to determine the approximate age of primary infection, which could inform the optimal vaccination window and identify the best target populations for CMV vaccine in developing countries. To determine CMV antibody kinetics across time, we measured CMV-specific antibodies in serum samples collected longitudinally in the first 15 years of life from two rural areas in Kilifi, Kenya. Understanding CMV immunity in children is particularly important for several reasons. In sub-Saharan Africa and other LMICs, primary CMV infection typically occurs during infancy or early childhood, before adolescence or pregnancy, underscoring the need for early-life immunological surveillance. While CMV vaccines are under active development, most deployment strategies focus on reproductive-age populations, despite the fact that infection often precedes this period in high-transmission regions[11,13]. The longitudinal data on CMV antibody dynamics in African children reported here sought to address this gap by leveraging over a decade of follow-up in two well-characterized Kenyan cohorts to assess the temporal patterns of antibody acquisition in early life.

Methodology

Study design and setting

This study was nested within the Kilifi Health and Demographic Surveillance System (KHDSS), a population-based surveillance platform covering approximately 300,000 residents in Kilifi County, Kenya[17]. The KHDSS provides longitudinal demographic and clinical surveillance, linking household data with health facility records using unique person identifiers. The present analysis utilized data and archived serum samples from two population-based pediatric cohorts - Ngerenya and Junju - enrolled between 1998 and 2017 as part of annual malaria cross-sectional surveys.

Participants and source population

Children were eligible for enrolment in the parent malaria surveillance cohorts if they were residents of the KHDSS, aged under 15 years at the time of enrolment, and had no acute illness at the time of the annual survey. Participants were recruited through community engagement and household enumeration exercises. The total source population across both cohorts exceeded 3,000 children over the study period.

For the present analysis, we selected a subset of 123 children based on the following inclusion criteria: (i) availability of at least eight annual serum samples, and (ii) minimal missing data across the sampling timeline. This subsample was chosen to enable robust modelling of individual-level antibody dynamics and was not selected based on any immunological or clinical outcome. This approach minimised the risk of selection bias.

Exposure setting and follow-up

Ngerenya and Junju differ markedly in malaria transmission intensity. Ngerenya experienced a substantial decline in Plasmodium falciparum transmission beginning in the early 2000 s, while Junju remained moderately endemic, with parasite prevalence averaging ~ 30% during rainy seasons. Children were visited weekly at home by fieldworkers for fever surveillance. Febrile episodes were confirmed using rapid diagnostic tests (RDTs) and microscopy, and clinical malaria episodes were treated according to national guidelines.

Sampling and health status

Serum samples were collected annually from all children during scheduled household visits. Sampling was restricted to children who were well at the time of visit, with no febrile illness or acute symptoms. No blood was drawn during episodes of illness. All samples were collected under aseptic conditions and processed using standardised protocols, then stored at – 80 °C for future immunological analysis. All serum samples used in this analysis were drawn from home-based visits, not clinic settings.

Linkage to hospital and clinical data

Through the KHDSS person-identifier system, we tracked all hospitalisations and outpatient visits during the follow-up period for each child. None of the hospitalisation records for the 123 children analysed were attributable to CMV-related illness, suggesting that observed antibody dynamics were not influenced by symptomatic CMV disease.

Ethical approval

The study was conducted according to the principles of Good Clinical Laboratory practice and the Declaration of Helsinki. Ethical approval was obtained from the Scientific Ethics Research Unit (SERU)of the Kenya Medical Research Institute (KEMRI). Written informed consent was obtained from the legal guardians of all study participants.

CMV antibody longevity was assayed at each time point for every study participant in both cohorts using direct antibody immunoassays on protein microarrays. The data are presented in anonymous codes delinked from the participant's identifiable data.

Antibody assays The CMV tegument phosphoprotein (pp150, supplier (MyBioSource; cat no. MBS319098) was reconstituted in a buffer comprising of glycerol in distilled water and 1% triton 100. This antigen, along with other infectious disease antigens that are not included in this report (including malaria, measles, rubella and influenza), were deposited onto Epoxy-coated glass slides by non-contact printing using the Marathon Argus microarrayer (Arrayjet, Scotland). Printed slides were coupled onto hybridization cassettes and washed thrice using the 1X PBST (Phosphate Buffered Saline and 0.05% Tween-20). The slides were then incubated for one hour at 37 °C with a blocking buffer (PBST and 5% BSA). Sera were diluted 1:30 in PBST & 5% BSA, then loaded onto slides and incubated on a shaking platform at room temperature for three hours. The slides were then washed as above and incubated with secondary antibodies as follows: goat anti-human IgG conjugated to Alexa

flour 647 (SouthernBiotech, cat no. 2040-3) and goat anti-human IgA conjugated to Alexa-fluor 555 (Southern biotech, cat no.2050-32) for one hour 30 min at 25 °C. Next, the slides were washed as before, disassembled from the hybridization cassettes, rinsed using Milli-Q water, and scanned on the GenePix 4300 A scanner (635 nm at PMT 600 and 532 nm at PMT 700). The generated data was imported into R software (v4.4.2) for analysis.

Data analysis Data analysis was done using R software (v4.4.2). Mean antibody levels (expressed as MFIs) for each sample were calculated from the quadruplicate spots, spatially distributed on two separate mini arrays and used to plot CMV antibody kinetics. We used piecewise mixed effects models to estimate individual-level trajectories and population-level inflection points.

Results

We measured antibodies against the CMV tegument phosphoprotein (pp150) in a total of 1,212 serum samples from 123 individuals who were followed up for a median duration of 10 years (range: 7–14 years), representing a total of 1,392 years of observation. Study participants from the Ngerenya cohort were followed up from 2002 to 2017 while those from the Junju cohort were followed up from 2007 to 2017 (Table 1). A summary of the sampling frames for both cohorts is shown in Supplementary Fig. 1.

The long-term temporal dynamics of CMV-specific antibodies were characterized by assessing age-specific variation in antibody levels against pp150. Antibody data from the Ngerenya cohort was partitioned into 18 age strata, while the Junju cohort was divided into 16 strata, each of which represented a year since recruitment (Fig. 1). In both cohorts, we observed a stereotypical pattern of antibody acquisition and loss at the population level: a rapid early phase of antibody acquisition that peaked at about four years of age was followed by a subsequent phase that was marked by a progressive loss of antibody (Fig. 1). Each cohort's antibody trough was evident at different ages (approximately 8 and 10 years of age for Ngerenya and Junju respectively), and a second phase of antibody re-acquisition followed soon afterwards.

Since this phased pattern of antibody acquisition and loss at the population level suggested that protective humoral immunity against CMV re-infection was relatively short-lived, we sought to estimate the effective duration of protection from re-infection using segmented regression modeling. We focused on the Ngerenya cohort for this analysis due to its low level of transmission of malaria parasites, which are known to attenuate the host's immune response to infection. We tracked the progressive loss of pp150-specific antibodies using unsupervised piecewise regression following an earlier acquisition phase. We found an inflection point at 7.57 years of age, which marked the end of the antibody decay phase and the start of a period of antibody re-acquisition (Fig. 2).

We then visualized pp150-specific antibody trajectories at the individual level over the first 10–14 years of life in order to determine if the dynamics of individual antibodies generally matched those of the population. For most children, we found an extremely early peak in pp150-specific antibodies, which was followed by a period of sustained decline in the ensuing years, as shown by the four examples in Fig. 3. For almost all the children analysed, this gradual decline was periodically interrupted by a spike in pp150-specific antibodies, followed by a further period of antibody decline.

Discussion

This study provides insights into the long-term dynamics of CMV-specific antibodies from childhood to early adolescence in a rural Kenyan setting. We were able to identify different stages of antibody acquisition, decay, and re-acquisition by monitoring antibodies against the CMV tegument phosphoprotein (pp150) in two cohorts. The observed early peak in antibody levels, which occurred in the first few years of life in both cohorts, aligns with previous studies that highlight early CMV exposure in low and middle-income settings. Previous studies suggest that CMV transmission occurs predominantly through close contact in childhood, particularly in low-income settings where household density and childcare practices facilitate viral spread[18,19]. The rapid acquisition of antibodies in our cohorts reinforces the role of early childhood as a critical window for primary CMV infection in settings such as ours. While the serological nature of the data does not allow us to directly distinguish between primary infection, reinfection, or reactivation, the longitudinal patterns observed offer a valuable window into CMV immune exposure in early life.

While pp150 is not a known correlate of protective immunity, its temporal dynamics offer a useful proxy for tracking long-term humoral responses to CMV exposure. The phased decline in antibody levels observed during mid-childhood provides insight into the waning of CMV-specific antibodies over time. Notably, this decline was frequently interrupted by periodic spikes in pp150-specific antibodies—patterns that may reflect natural immune boosting events, potentially due to subclinical reactivation or reinfection, though our study cannot distinguish between these possibilities. These boosting episodes appeared to sustain antibody levels above the seronegativity threshold across the entire cohort. The segmented regression analysis revealed a consistent inflection point at 7.57

	Junju	Ngerenya
No of children in cohort	58	64
Duration of follow-up	2007–2017	2002–2017
Average number of samples study subject (range)	8 (7–10)	11 (8–14)
Total Serum Samples tested	505	707

Table 1. Demographic characteristics of the longitudinal study cohorts.

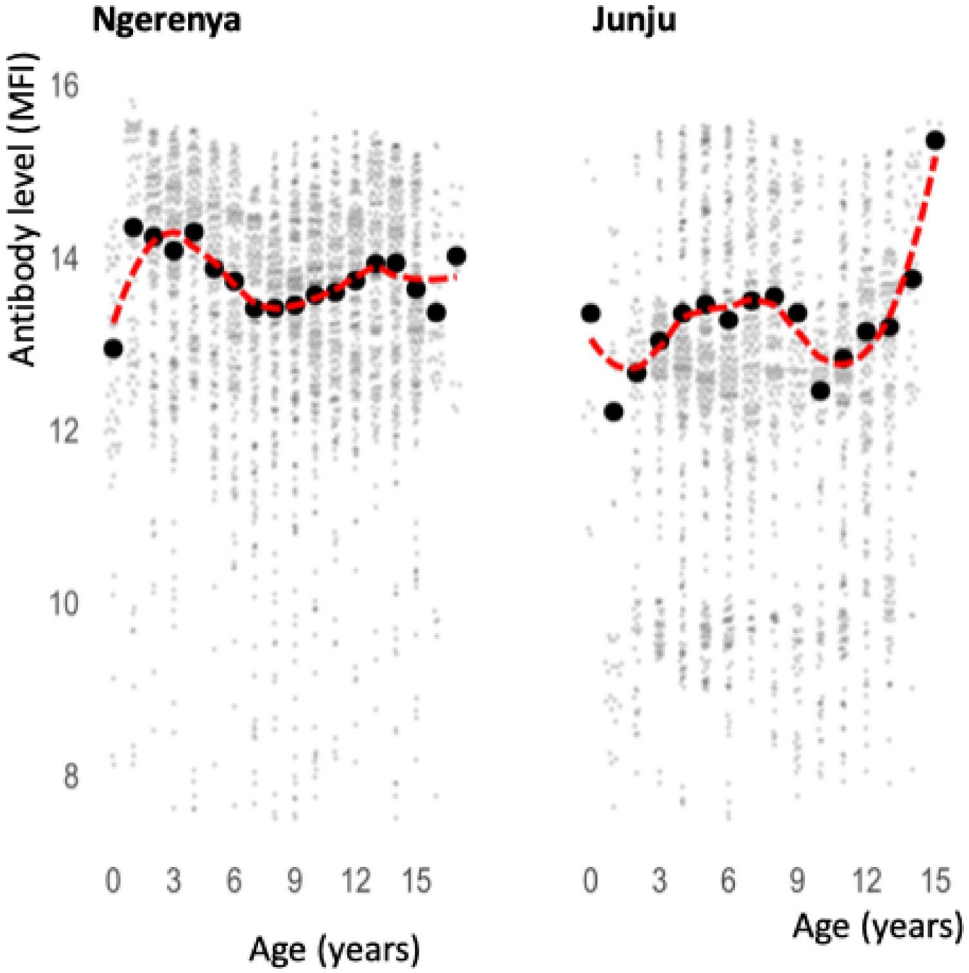

Fig. 1. Dynamic trends of population-level CMV pp150 antibody acquisition and decay in the Ngerenya and Junju cohorts. The X-axis represents the age in years and the y-axis shows CMV antibody measurement in mean fluorescent intensity (MFI) values. The red dotted line represents the smoothed trend of antibody levels over time.

years in the Ngerenya cohort, marking a transition from antibody decline to re-accumulation. This shift likely reflects ongoing CMV exposure in a population where infection is typically acquired early in life, underscoring the importance of understanding CMV immunodynamics throughout childhood in high-transmission settings.

The implications of our findings for CMV vaccine development are significant. The rapid acquisition of antibodies during early childhood suggests that vaccination efforts should target infants and young children in settings such as ours before the peak period of primary infection. Our results also highlight the importance of considering environmental factors, such as malaria co-endemicity, when designing vaccination strategies for resource-limited settings. Future research should build on our findings by incorporating cellular immunity data to provide a more comprehensive understanding of CMV-specific immune responses. Additionally, exploring the role of maternal antibodies and their transfer to infants could shed light on the early dynamics of CMV infection. Longitudinal studies in other geographic regions with varying transmission intensities would also help validate and extend our observations.

This study has several limitations. First, while pp150 is a conserved and immunogenic component of the CMV virion, it is not a validated correlate of protective immunity. Our choice to focus on pp150 was driven by its strong and durable immunogenicity during natural infection, making it suitable for tracking long-term exposure, but not for inferring susceptibility to reinfection or immune protection. Second, we lacked virologic confirmation (e.g., CMV PCR or viral culture) and were therefore unable to distinguish whether observed antibody fluctuations reflected subclinical reinfection, reactivation, or nonspecific immune modulation. Third, antibody quantification was performed using a single serum dilution, which limits the precision of absolute titer estimation, though the large sample size and consistent protocol allowed robust inference of relative temporal patterns. Lastly, while we accounted for follow-up density by restricting analysis to participants with repeated samples, changes in cohort composition over time may still have influenced population-level trends. These limitations notwithstanding, the longitudinal design, decade-long follow-up, and use of high-throughput multiplex serology provide a rare and valuable window into CMV immune dynamics in early life in a high-transmission setting.

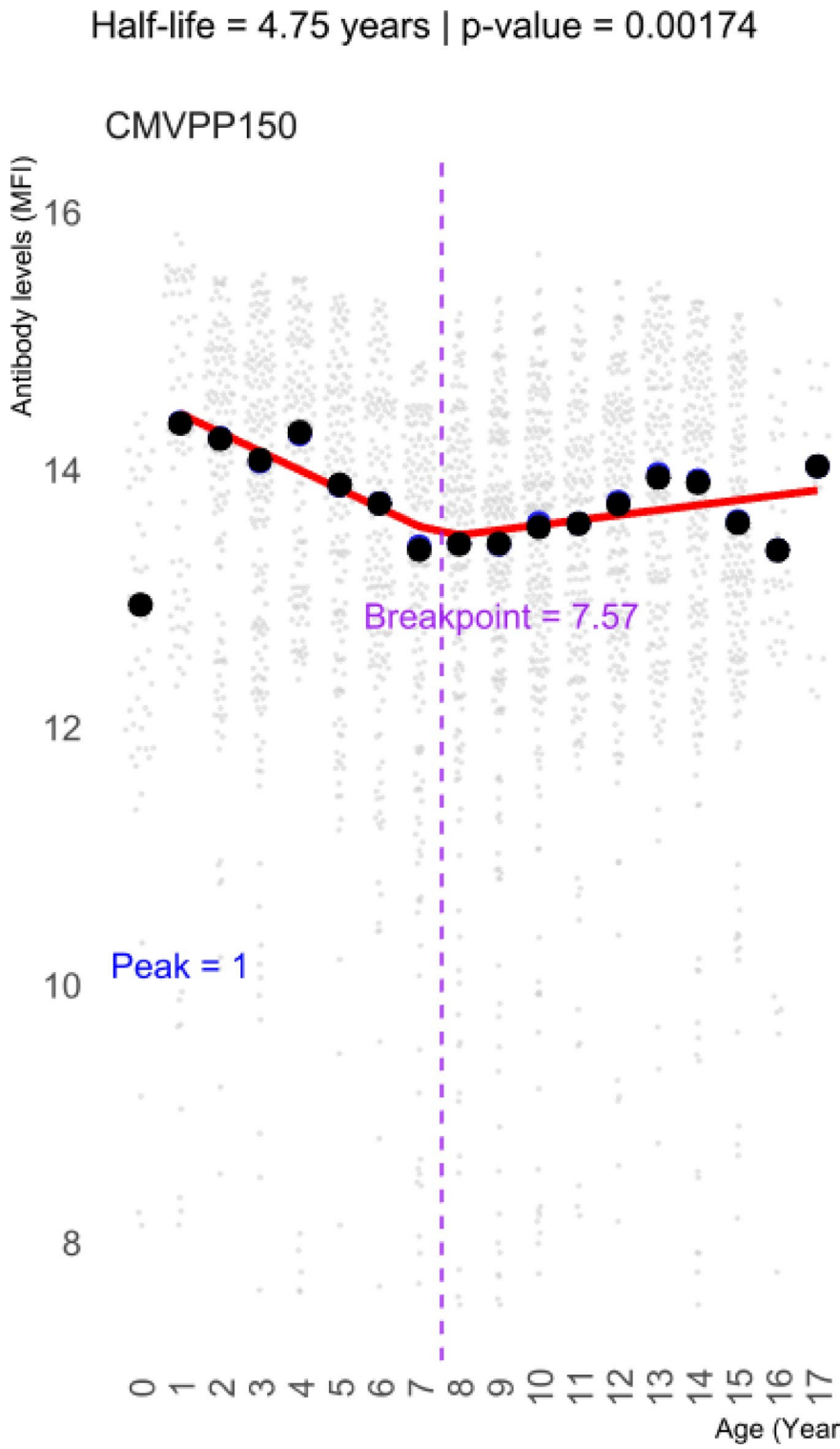

Fig. 2. Segmented logistic regression depicting the relationship between age and CMV pp150-specific antibody. Age-specific antibody data was segmented at specified age cutpoints, and linear regression models were fitted for each segment. The segments were then splined to visualize the relationship between age and antibody titers. An inflection point was identified at about 7.5 years of age, marking the end of a phase of antibody decline, followed by an antibody re-acquisition phase at the population level.

In conclusion, this study provides novel insights into the long-term dynamics of CMV-specific antibodies in a rural African population. The phased pattern of antibody acquisition, decay, and re-acquisition highlights the complexity of CMV immunity. These findings have important implications for CMV vaccine design and implementation in high-burden settings.

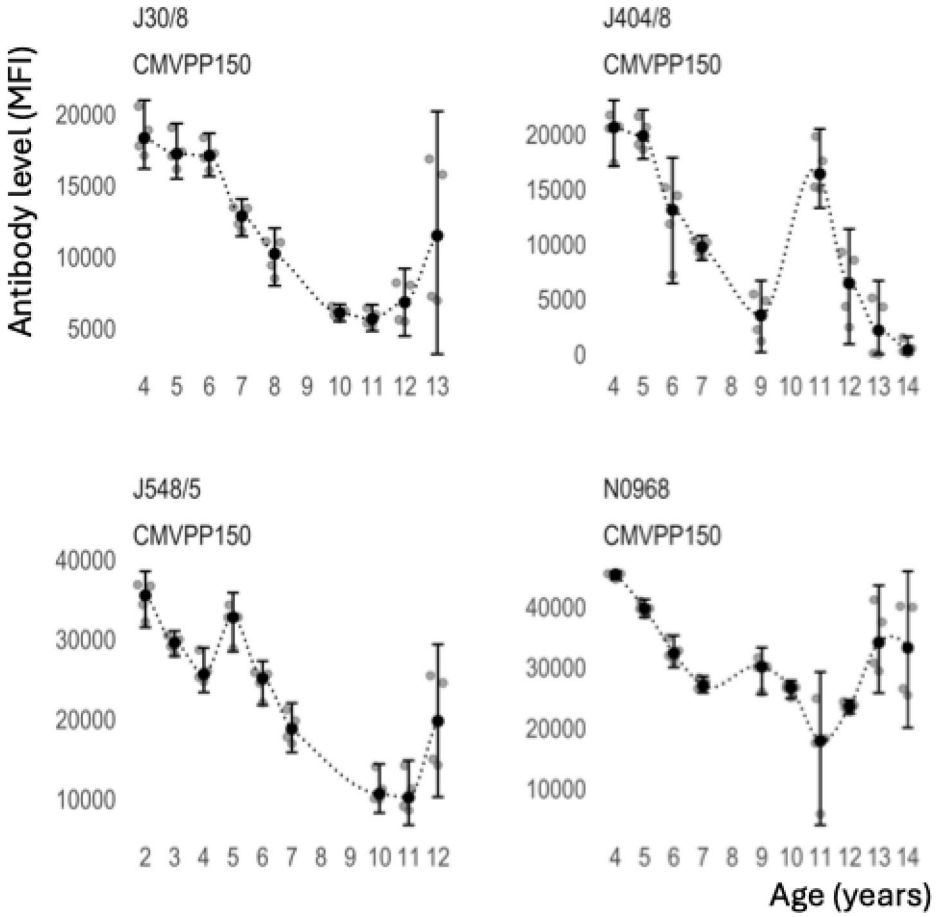

Fig. 3. Individual-level CMV antibody kinetics for four study participants (J30/8, J404/8, J548/5, and N0968). CMV antibody levels were measured as Median Fluorescence Intensity (MFI). The X-axis represents age in years, and the y-axis represents the mean MFI values.

Data availability

The datasets and R scripts used for the analysis in this study are available from the corresponding author upon reasonable request. Due to local laws and regulations, individual-level data from the Kilifi Health and Demographic Surveillance System (KHDSS), cannot be made publicly available. All data processing and visualization were performed using open-source R packages; package versions and analysis pipelines are available upon request.

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Author contributions

Project was conceived and designed by CJS. Laboratory processing of the samples was done by ETG, TOM, MWM, OKN and MSS. Data analysis and management was carried out by TO, MSS, ELG, CJS and MWM. MWM, TOM and MSS wrote the initial draft and ETG, OKN, TCK, CJS and DJF reviewed the manuscript and produced the final draft. All authors contributed to and reviewed the final draft.

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Declarations

Competing interests

The authors declare no competing interests.

Conflict of interest

The authors declare no competing interests.

Additional information

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