# LONGITUDINAL CORONAVIRUS ANTIBODY RESPONSES IN AFRICA BETWEEN 2013 AND 2021

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## ABSTRACT

Pre-existing coronavirus-specific antibody responses may affect SARS-CoV-2 responses. We evaluated longitudinal samples obtained before and during the pandemic in participants from Kenya, Nigeria, Tanzania and Uganda; 90% were people living with HIV.

Serum samples were tested using a multiplex bead-based immunoassay to measure antibody binding against 22 antigens including Nucleocapsid (N) and Spike (S) proteins of the 7 human coronaviruses and one malaria antigen.

We tested 819 longitudinal samples from 80 participants collected between July 2013 and May 2021 (3-16 samples per participant). Using a signal to noise ratio (S/N) >10, 13, 1, and 5 participants showed at least one time point with IgG responses to S of SARS-CoV-2 (ancestral), SARS-CoV-1 and MERS-CoV respectively while 14, 8, and 11 participants showed responses to N before 2020. Across individuals, IgG binding to SARS-CoV-2 S subunit S2 was most frequently detected and it showed the highest within-host fluctuations over time. A few individuals had elevated responses that persisted over years towards multiple antigens, most frequently to different SARS-CoV-2 antigens and rarely to distinct viruses. One individual showed high RBD-specific IgG responses to distinct coronaviruses at a single time point before 2020. Responses against coronaviruses measured post-2020 generally correlated with responses measured before 2020, except for a subset of infected individuals whose responses against SARS-CoV-2 dramatically increased post-pandemic. IgG responses against the ancestral SARS-CoV-2 variant were most correlated with responses against Alpha and Gamma (then to Beta and Delta, rho >0.75) variants. Using an IgM S/N >10, 31 participants were Malaria positive and 22 showed concurrent elevated coronavirus IgM responses. However, about half of the malaria positive participants had no IgG responses against any coronavirus antigen and the rest presented limited and variable patterns of association between responses against coronaviruses and malaria.

Our study confirmed that a small subset of individuals in Africa had long-lasting IgG coronavirus-specific antibodies before the pandemic. While there was an association between coronavirus IgM responses and responses against malaria, there was no correlation between IgG responses and malaria infection. Further analysis is needed to better understand the interactions between antigens in the development of antibody immunity to coronaviruses.

## BACKGROUND

We observed significantly higher binding antibody responses to coronavirus in Africa than in Thailand before the COVID-19 pandemic in a previous study (Li et al., 2022). To further understand these antibody responses, we evaluated longitudinal samples obtained before and during the COVID-19 pandemic in participants from Kenya, Nigeria, Tanzania and Uganda.

## METHODS

**Multiplex bead-based immunoassay**

- 819 longitudinal samples from 80 participants
- Antibody responses against 22 human coronavirus antigens and one malaria antigen and 4 detection reagents (IgG, IgA, IgM, FcgR2B) were measured

| | SARS-CoV-2 | | | | | SARS-CoV-1 | MERS-CoV | HKU1 | OC43 | 229E | NL63 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wuhan-Hu-1 | Alpha | Beta | Delta | Gamma | | | | | | |
| Spike Trimer | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| RBD | ✓ | | | | | ✓ | ✓ | | | | |
| S2 | ✓ | | | | | | | | | | |
| N | ✓ | | | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

- Longitudinal SARS-CoV-2-like responses detected before the pandemic in a small set of participants in Africa
- Association between malaria infection and IgM responses against coronaviruses but no association with IgG responses

## RESULTS

**Table 1: Characteristics of the study participants**

| | Kenya | Nigeria | Tanzania | Uganda |
|---|---|---|---|---|
| Total N | 34 | 6 | 9 | 31 |
| Number of PLWH | 32 | 6 | 8 | 26 |
| Number of female | 17 | 4 | 5 | 13 |
| Age at first visit (Average) | 41 | 38 | 45 | 42 |
| Years in the study (Average) | 5.17 | 5.97 | 3.29 | 5.5 |
| Median days between two visits | 185 | 194 | 193 | 183 |

### Coronavirus-specific responses detected in pre-pandemic samples

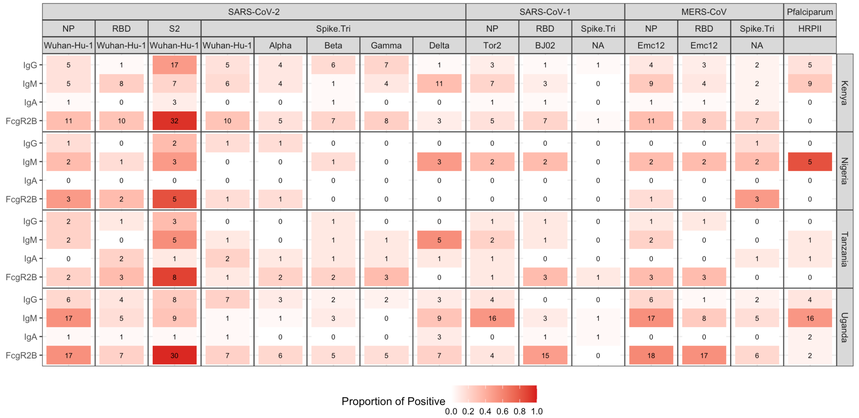



**Fig. 1: Proportion of participants with at least one coronavirus positive response pre-pandemic.** Number of positive participants in each country using S/N>10 against three outbreak coronaviruses and one malaria antigen across 3 isotypes and FcgR2B.

### IgG responses reflected phylogenetic distances between coronaviruses

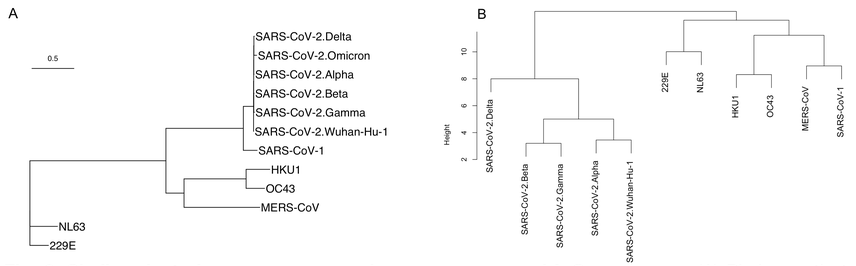



**Fig. 2: Similar clustering across coronavirus sequences and IgG responses.** (A) Phylogenetic tree based on Spike sequences. (B) Hierarchical cluster analysis of Euclidean distances in IgG responses against coronavirus Spike antigens using the earliest sample of each participant.

### Similar coronavirus responses before and after 2020

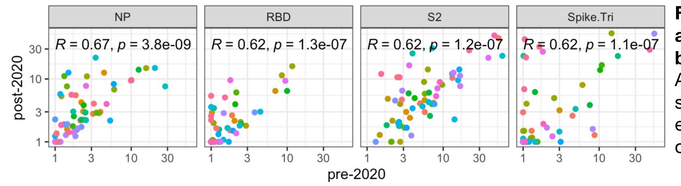



**Fig. 3: IgG responses against SARS-CoV-2 before and after 2020.** Average S/N were shown in the plots and each color represents one participant.

### Relationship between coronavirus responses and malaria infection

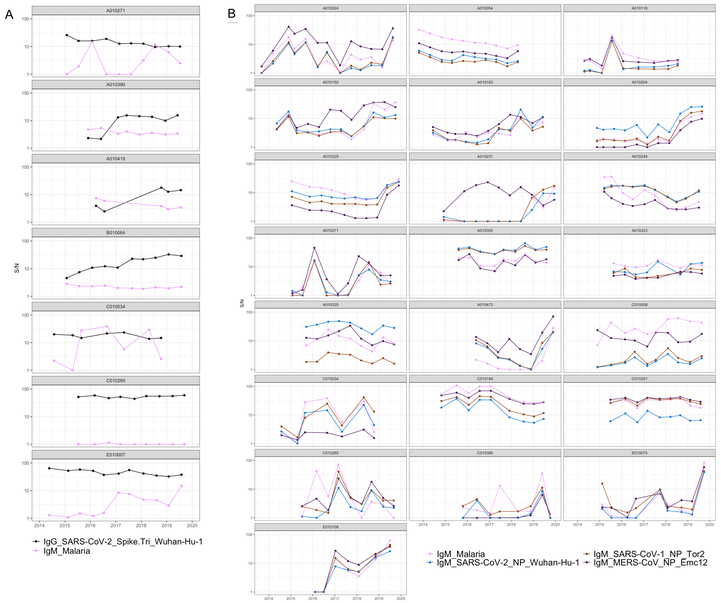



**Fig. 4: Longitudinal responses against coronaviruses and malaria reactivity.** Malaria reactivity was defined as S/N>10 in IgM responses against a malaria antigen. (A) Seven participants with persistent high IgG SARS-CoV-2 Spike responses showed different patterns of malaria responses. (B) Participants with malaria reactivity showed concurrent IgM coronavirus N responses.

## CONCLUSION

- Longitudinal antibody profiles showed long-lasting reactivity to outbreak coronaviruses among African participants pre-pandemic
- Distances between Ab binding responses matched phylogenetic distances between coronaviruses
- Responses before and after 2020 were correlated except for a subset of individuals whose responses against SARS-CoV-2 increased post-pandemic due to infection or vaccination
- Concurrent IgM reactivity against coronaviruses N was observed in participants with malaria reactivity but not linked to IgG responses
- Better understanding cross-reactivity with SARS-CoV-2 could provide insight on the interactions between COVID-19 and malaria infections

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