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Seroepidemiology of COVID-19 in pregnant women and their infants in Uganda and Malawi across multiple waves 2020-2022

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Abstract

Data on SARS-CoV-2 infection in pregnancy and infancy has accumulated throughout the course of the pandemic. However, limited information is available from countries in sub-Saharan Africa (SSA). Evidence regarding asymptomatic SARS-CoV-2 infection and adverse birth outcomes are also scarce in these countries. The pregnant woman and infant COVID in Africa study (PeriCOVID Africa) is a South-South-North partnership involving hospitals and health centres in five countries: Malawi, Uganda, Mozambique, The Gambia, and Kenya. The study leveraged data from three ongoing prospective cohort studies: Preparing for Group B Streptococcal Vaccines (GBS PREPARE), SARS-CoV-2 infection and COVID-19 in women and their infants in Kampala and Mukono (COMAC) and Pregnancy Care Integrating Translational Science Everywhere (PRECISE). In this paper we describe the seroepidemiology of SARS-CoV-2 infection in pregnant women enrolled in sites in Uganda and Malawi, and the impact of SARS-CoV-2 infection on pregnancy and infant outcomes.

The PeriCOVID study is a prospective mother-infant cohort study that recruited pregnant women at any gestation antenatally or on the day of delivery. A nasopharyngeal swab was taken from mothers at enrolment for RT-PCR confirmation of SARS-CoV-2 infection, and maternal and cord blood samples were tested for SARS-CoV-2 antibodies using Wantai and Euroimmune ELISA. The primary outcome was seroprevalence of SARS-CoV-2 antibodies in maternal blood, reported as the proportion of seropositive women by study site and wave of COVID-19 within each country. Placental transfer of antibodies was described using the geometric mean ratio (GMR). We also estimated the proportion of asymptomatic or subclinical COVID-19 infections in pregnant women using serological testing and collected

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adverse pregnancy and infancy outcomes (e.g. still-birth, prematurity, maternal or infant death).

In total, 1379 women were enrolled, giving birth to 1387 infants. Overall, 63% of pregnant women had a SARS-CoV-2 positive serology. Over subsequent waves (delta and omicron) , in the absence of vaccination, seropositivity rose from 20% to over 80%. The placental transfer GMR was 1.7, indicating active placental transfer of anti-spike IgG. There was no association between SARS-CoV-2 antibody positivity and adverse pregnancy or infancy outcomes.

This study describes the increasing prevalence of SARS CoV-2 antibodies in pregnant woman in Uganda and Malawi across waves of SARS-CoV-2 infection. Our study adds to existing evidence that suggests under-reporting of infection if based solely on cases with clinical disease, or a positive RT-PCR for SARS-CoV-2, as most of the women in our study had asymptomatic infections and did not seek medical care. This has implications for screening in subsequent outbreaks and pandemics where protection of pregnant women and effect of infection in pregnancy on the infant are unknown.

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Introduction

The initial predictions of the impact of SARS-CoV-2 in sub-Saharan Africa suggested high case numbers and fatalities (1), yet there is evidence to suggest that the pandemic evolved differently in Africa than in other regions (2, 3). Several factors have been proposed to explain the relatively low frequency of severe SARS-CoV-2 illness, including a younger population, a lack of long-term care facilities and reduced population density (4-7). However, limited testing capacity and weak reporting structures in many sub-Saharan African countries may also result in under-reporting, leading to an underestimation of the true risk of serious SARS-CoV-2 infection (3). Infection in pregnancy, even if asymptomatic or mild, may have long-term impacts for a pregnant woman or impair the neurodevelopment of her child, a risk which is well established for viral infections such as Zika virus or cytomegalovirus (8).Data on the impact of SARS-CoV-2 infection in pregnancy on neurodevelopmental outcomes is emerging (9, 10). Evidence regarding asymptomatic SARS-CoV-2 infection and adverse birth outcomes is limited and the long-term effects of the pandemic on infant health remain poorly understood (11).

Serological surveillance is a useful means of estimating population-level immunity against infectious diseases using cross-sectional studies of antibody prevalence (12). In the case of SARS-CoV-2, serological surveys are helpful in estimating the number of people who have been exposed to SARS-CoV-2, whether they were symptomatic or not to better clarify the dynamics of exposure during the different epidemic waves as vaccines were rolled out (13). For example, seroprevalence surveys conducted across Kenya, South Africa and Malawi

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have all reported community transmission, which is several times higher than that detected by national virological surveillance programmes (14-16).

Seroprevalence studies in pregnancy enables insights into the real magnitude of exposure to SARS-CoV-2 infections and the extent of under-reporting of SARS-CoV-2 cases. Information on seroprevalence in pregnancy, placental antibody transfer and antibody half-life also offer the possibility to approximate the number of mother-infant dyads who could potentially exhibit immunological protection against subsequent infections, especially with low vaccine coverage in many low-resource settings (LRS). Finally, seroprevalence studies can also provide insight into the relationship between infection and vaccination, symptoms, and antibody responses to assist with future screening and prevention policies in pregnancy.

To address these specific gaps, we investigated the seroprevalence and the associations of different factors on seropositivity to the SARS-CoV-2 virus among pregnant women and their infants in Uganda and Malawi. This was performed during consecutive SARS-CoV-2 waves.

Methods

Study design and participants

PeriCOVID Africa is a multi-site prospective mother-infant cohort study using an adapted WHO UNITY protocol (17), whereby women were categorised into two categories dependent on serological testing using the Wantai total antibody assay as exposed (positive serology) or unexposed (negative serology) to SARS-CoV-2. Women were additionally screened for symptoms, using the WHO definition for probable COVID-19 disease at the

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time of study participation (18). We defined symptomatic COVID-19 infection according to the WHO definitions of probable COVID-19 illness (18) and asymptomatic infection as seropositive or PCR positive at enrolment in the absence of reported symptoms. Unexposed women were those with no reported symptoms consistent with SARS-CoV-2 infection and negative serology.

Recruitment

Each study adapted the WHO UNITY protocol according to local needs and capacity considerations. Women were recruited into the study either during an antenatal visit, or during labour at seven study clinics and hospitals in Uganda and Malawi. In all studies, gestational age at enrolment was estimated by date of last menstrual period and fundal height. Additionally, in periCOVID Uganda and periCOVID Malawi Ballard scores were calculated at birth. Individual study recruitment, sampling and follow up are shown in Table 1. The first participant was recruited 1st February 2021. Final participant follow and sampling was concluded by 31st January 2022.

Table 1. Recruitment, sampling and follow-up by study site.

Study Site	Enrolment timing	Study period	Inclusion Criteria	Exclusion Criteria	Sampling at enrolment	Follow up
All sites			Willingness to provide informed consent		Maternal and Cord blood	6 week follow up
periCOVID Uganda	Antenatal clinic or delivery	February 2021 – January 2022	Pregnant women (including emancipated minors aged over 14 years) at any gestation including the day of delivery Planning to deliver at one of the designated study sites and willing to stay in the area for the first six weeks of their baby's life Willing to attend a follow up visit at six weeks postpartum	No exclusion criteria	Maternal nasopharyngeal swab*	**For COVID-19 Cases only*. Maternal:** nasopharyngeal maternal blood sample (5ml serum), **Infant**: nasal swab, blood sample (2-5ml) or dried blood spot,
COMAC	Delivery	August 2021 – January 2022	- low risk of infection with tuberculosis in the household -mother of legal age (including if emancipated minor) for participation -mother residing within the study area, not intending to move out	- Baby weighs less than 2kg at birth - Baby requires hospital admission for severe illness at birth - Serious congenital malformation(s) - severely ill mother on the day of giving birth whose condition(s)	Nil additional	**Maternal** blood test at 14 weeks. Nasopharyngeal swab at 6 weeks and 14 weeks. **Infant**: blood test at 14 weeks. Nasopharyngeal swab at 6 weeks and 14 weeks

			of the area in the next 4 months and is likely to be traceable for up to 12 months. - HIV-1 positive women should be receiving the necessary antiretroviral treatment and prophylaxis (Ugandan Option B+ guidelines) HIV-exposed babies receives peri exposure prophylaxis (Ugandan Option B+ guidelines)	require(s) hospitalization -		
Malawi	Delivery	March 2021 – January 2022	All women presenting to QECH in labour with an estimated gestation of 28 weeks or greater who - Are willing to attend a follow up visit at 6 weeks postpartum	No exclusion criteria.	Nil additional	**For COVID-19 cases only.** M**aternal** *blood, rectal swab, breastmilk. I**nfant*:** blood

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COVID-19 testing

All women at all study sites were screened for COVID-19 symptoms using a standardised data collection form with questions including a recent history of fever, cough, anosmia and ageusia and contact with a known SARS-Cov-2 case. Information on COVID-19 illness symptoms was collected at enrolment for the 14 days prior to enrolment in PeriCOVID Uganda and Malawi, and in the 28 days prior to enrolment in COMAC Uganda. Women enrolled in PeriCOVID Uganda had a nasal swab taken at enrolment to test for SARS-CoV-2 by PCR. In Malawi throat swabs were taken if the clinical syndrome was suggestive of a probable COVID-19 illness as defined by the WHO (18).

Blood sampling

Sampling at all sites for antibodies to SARS-COV-2 included at least a maternal venous and paired cord blood sample (see Table 1 for sampling schedule at each site).

Data collection

Each site (KNRH, Kawaala, Kitebi or Mukono General Hospital in Kampala, Uganda and QECH in Blantyre, Malawi) used a study questionnaire which was completed by research staff to capture information from study participants and then uploaded to a central RedCAP database. This included data on maternal age, significant past medical history, HIV and socioeconomic status; onset and duration of signs and symptoms of SARS-CoV-2 illness if present, and self-reporting of prior SARS-CoV-2 illness; gestational age at enrolment, parity, number of foetuses (if known before delivery), co-infection with malaria, vaccinations received in pregnancy including the SARS-CoV-2 vaccines; gestational age at delivery, delivery method, intrapartum and postpartum complications such as pre-term birth,

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stillbirth, abortion, and maternal death; neonatal outcomes including evidence of COVID-19 illness, Neonatal Intensive Care Unit (NICU) admission, low birth weight and neonatal death; infant health status

Laboratory methods

As per FIND guidelines at the time of the study protocol development (19), we used three different SARS-CoV-2 specific antibody assays that targeted either the receptor binding domain (RBD; total antibody), the spike protein (anti-S, total antibody) or nuclear capsid (anti-NCP, IgG). We performed in-house specificity and sensitivity testing, respectively, using 100 pre-COVID19 (pre-2019) samples selected by month for seasonality assessment and 20 PCR positive samples (19) to perform assay validation. We also examined potential cross-reactivity in our assay from malaria-specific antibodies using 74 women who tested positive to malaria (antibody positive by rapid diagnostic test (RDT) from pre-COVID samples and 15 SARS-COV-2 PCR positive samples in women who did not have malaria (negative RDT) during the COVID-19 pandemic. Results can be seen in Supplementary Table S1.

Laboratory analysis

Laboratory testing for SARS-COV-2 antibodies was performed at the MRC/UVRI and LSHTM Uganda Research Unit or the Malawi Liverpool Wellcome facilities using the Wantai SARS-CoV-2 total antibody ELISA kit (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd, Beijing, China). The manufacturer-reported assay sensitivity is 94.4%, with a specificity of 100%. All specimens that tested positive for Wantai were tested using Euroimmun Anti-SARS-CoV-2 NCP/S ELISA (IgG) (Euroimmun, Lübeck, Germany) kits for the detection of IgG antibodies to

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SARS-COV-2 nucleocapsid and spike (S1 and S2) proteins, respectively. Euroimmun Anti-SARS-CoV-2 NCP/S ELISA (IgG) is a semi-quantitative immunoassay with a reported sensitivity of 94.6% and specificity of 99.8% in samples collected at least 10 days after confirmed SARS-COV-2 infection. A sample was considered positive if the Wantai test was positive. Results which were reported as borderline on the Euroinmmune assay were considered as negative for the purpose of our analysis. Due to variable specificity of the Euroimmune assay, we report Wantai results for all outcomes as per manufacturer's instructions.

As the Wantai ELISA is a qualitative test, WHO standards for NCP and S proteins were run on all assays. The stock concentration for NCP and S proteins was 123µg/ml and 1000µg/ml, respectively. The working concentration for both NCP and S proteins was 2 µg/ml. The calibration curve was created using WHO International standard for anti-SARS-CoV-2 immunoglobulin (NIBSC 20/136) using a 12-well dilution series created in 1.75-fold steps, starting at 1:200 and this series was used to generate the curve. All laboratory testing in Uganda for SARS-CoV-2 antibodies was performed using the ETI-MAX 3000 (Diasorin, Saluggia, Italy).

Data Analysis

Although there was no predefined sample size, we aimed to obtain samples from at least 1000 women and infants across all sites. The sero-epidemiological analyses were carried out using all participants with enrolment blood sample results available for analysis. The proportion of seropositive results was calculated for the individual waves of SARS-CoV-2 within each country. This was done for maternal blood samples to estimate the seroprevalence of SARS-CoV-2 among pregnant women, and for cord blood samples to

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estimate the seroprevalence of SARS-CoV-2 antibodies in infants. The dates used to define the SARS-COV-2 waves in each country are given in Table S2 and were taken from Our World in Data (20) . Waves were defined by taking the nadir between each peak for the start and end dates of each wave. Confidence intervals for prevalence estimates were computed using the Clopper-Pearson (Binomial Exact) method.

The geometric mean concentration (GMC) and 95% confidence intervals (CI) of anti-S and anti-NCP antibodies measured on the Euroimmune assay were calculated for mother-infant pairs. To study the rate of placental transfer of SARS-CoV-2 antibodies, the geometric mean ratio (GMR) of infant to maternal antibodies was calculated.

The proportion of participants with a symptomatic or asymptomatic infection was calculated for women who were seropositive at enrolment and for those who had a positive RT-PCR test at enrolment.

The impact of infection on key pregnancy and neonatal outcomes was modelled for women and infants in PeriCOVID Malawi and Uganda log-binomial generalised linear models (GLM) which were adjusted for country. Models were constructed for maternal death, infant death, combined adverse pregnancy outcome (at least one of maternal death, abortion, premature labour or stillbirth), and the combined adverse neonatal outcome (at least one of neonatal/infant death, prematurity, low birth weight, NICU admission after birth, or birth asphyxia). Relative risks were presented with 95% confidence intervals for all models reaching convergence. Results were not presented for adverse outcomes with fewer than 5 events.

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Statistical analyses were carried out using R version 4.2.1. No significance tests were conducted.

Ethical Considerations

The study documents were reviewed and approved by the Ethics Committee of the relevant institutions: Uganda: Makarere University School of Medicine (SOMREC), Uganda National Council for Science and Technology (UNCST); Regional Committees for Medical and Research Ethics in Norway; Malawi: College of Medicine Research Ethics Committee (COMREC).

Results

In total, 1379 women were enrolled, giving birth to 1387 infants (Fig 1, Table 2). The mean age of all women was 26 years (SD 6) across the three sites. Most (n=1346, 98%) pregnancies were singleton. The HIV prevalence was 9% (n= 272). 371 women delivered outside of a study hospital and so no blood samples were available for analysis. Deliveries of 1024 infants with cord blood samples occurred at study sites. Almost all were livebirths (n=1009/1024, 99%) and most (n=888/1024, 87%) were not admitted to the NICU after birth (Table 2). A total of 909/1379 (65.9%) women in periCOVID Uganda and periCOVID Malawi had a PCR result at enrolment available for analysis of which 68/909 (7.5%) women had positive PCR results for SARS-CoV-2. Amongst these women, 77.9% (n=53) had symptoms consistent with COVID-19 disease. The majority (88.7%, n=47/53) were in Malawi who were

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performing RT-PCR testing only on symptomatic women. In periCOVID Uganda, where all women had a RT-PCR test at enrolment, 31.6% (n=6/19) of those with a positive RT-PCR test had symptoms suggestive of COVID-19 disease (Supplemental Table 3). Genotyping revealed all positive cases from Uganda to be of the delta variant.

Fig 1. Study flow chart. Study flow chart to show the number of women enrolled by study, with maternal serology available at enrolment (maternal result) and the infants with cord blood serology (cord blood result) available.

257 **Table 2. Demographics for women and infants in the study.**
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Characteristic of women enrolled	Overall, N = 1,379	PeriCOVID Malawi, N = 387	PeriCOVID Uganda, N = 836	COMAC Uganda, N = 156
Age (years)	26 (6)	25 (7)	26 (6)	27 (5)
Missing	2	2	0	0
Number of fetuses				
Singleton	1,346 (98%)	371 (96%)	819 (98%)	156 (100%)
Twins	30 (2.2%)	14 (3.6%)	16 (1.9%)	0 (0%)
Triplets	1 (<0.1%)	0 (0%)	1 (0.1%)	0 (0%)
Missing	2	2	0	0
Highest level of education				
Graduate Education /Terminal Degree Completed.	23 (1.7%)	1 (0.3%)	20 (2.4%)	2 (1.3%)
No Formal Education	12 (0.9%)	5 (1.3%)	5 (0.6%)	2 (1.3%)
Other	2 (0.1%)	0 (0%)	2 (0.2%)	0 (0%)
Primary Education Completed	211 (15%)	98 (26%)	84 (10%)	29 (19%)
Secondary Education Completed	252 (18%)	129 (34%)	105 (13%)	18 (12%)

Some Primary Education	239 (17%)	126 (33%)	86 (10%)	27 (17%)
Some Secondary Education	509 (37%)	0 (0%)	432 (52%)	77 (49%)
University / College Completed	127 (9.2%)	25 (6.5%)	101 (12%)	1 (0.6%)
Missing	4	3	1	0
HIV				
No	1,107 (91%)	325 (84%)	782 (94%)	0 (NA%)
Yes	114 (9.3%)	60 (16%)	54 (6.5%)	0 (NA%)
Missing	158	2	0	156
Characteristics of infants born and providing a cord blood sample	**Overall, N = 1,024**	**PeriCOVID Malawi, N = 365**	**PeriCOVID Uganda, N = 503**	**COMAC Uganda, N = 156**
Status of infant at birth				
Livebirth	1,009 (99%)	357 (99%)	496 (99%)	156 (100%)*
Miscarriage	2 (0.2%)	1 (0.3%)	1 (0.2%)	0 (0%)
Stillbirth	10 (1.0%)	4 (1.1%)	6 (1.2%)	0 (0%)
Missing	3	3	0	0
Gestation at birth (weeks)	27, 44	27, 44	28, 44	Inf, -Inf
Missing	157	1	0	156

Sex				
Female	492 (48%)	164 (45%)	248 (49%)	80 (51%)
Male	531 (52%)	200 (55%)	255 (51%)	76 (49%)
Missing	1	1	0	0
Birth weight (grams)				
Mean (SD)	3,082 (540)	2,910 (573)	3,151 (504)	3,262 (458)
Range	600, 4,850	600, 4,500	1,200, 4,850	2,100, 4,700
Missing	8	2	6	0
Admitted to NICU after birth				
No	888 (87%)	264 (73%)	468 (93%)	156 (100%)*
Yes	133 (13%)	98 (27%)	35 (7.0%)	0 (0%)
Missing	3	3	0	0

* COMAC Uganda only recruited infants who were born alive and not admitted to the NICU after birth

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Seropositivity in pregnant women and their infants

Overall, 1371/1379 maternal samples (382 from Malawi and 989 from Uganda) and 987/1024 cord blood samples (359 from Malawi and 628 from Uganda) were available for analysis (Table 3 and 4). There were 875 SARS-CoV-2 seropositive women in the study (257 in Malawi, 618 in Uganda), of whom 791 (90.4%) were asymptomatic in the 14 days (periCOVID Uganda and Malawi) -28 days (COMAC) prior to enrolment. This corresponded in Malawi to 21 asymptomatic seropositive participants during the second wave, 134 during the third wave and 57 in the fourth wave. In Uganda, this corresponded to 92 in the first wave, 422 in the second wave and 65 in the third wave (Table 3).

There was an increase in seropositivity with each subsequent wave, and the majority (70-100%) of women with positive serology were asymptomatic in the 14 days prior to sampling. Fig 2 shows the monthly total antibody positivity rate for each site with the daily number of new cases per million in Uganda and Malawi. The concordance between maternal and cord samples was high (72-99%).

Fig 2. Monthly sero-positivity by country in pregnant women. Black line is the monthly proportion of results that were positive. Shaded in green is the proportion of Wantai positive samples that were also positive for anti-spike IgG in the Euroimmune assay. Blue line is the number of new cases per million in Malawi and Uganda, taken from Our World in Data.

286 Table 3 – Maternal results

	PeriCOVID Malawi			PeriCOVID Uganda		COMAC Uganda	
Characteristic	Wave 2, N = 51 N (%) (95% CI)	Wave 3, N = 229 N (%) (95% CI)	Wave 4, N = 107 N (%) (95% CI)	Wave 1, N = 194 N (%) (95% CI)	Wave 2, N = 642 N (%) (95% CI)	Wave 2, N = 82 N (%) (95% CI)	Wave 3, N = 74 N (%) (95% CI)
Maternal blood sample							
Negative	24 (51%) (36%, 66%)	75 (33%) (27%, 39%)	26 (24%) (17%, 34%)	102 (53%) (45%, 60%)	240 (38%) (34%, 41%)	22 (27%) (18%, 38%)	7 (9.6%) (3.9%, 19%)
Positive	23 (49%) (34%, 64%)	153 (67%) (61%, 73%)	81 (76%) (66%, 83%)	92 (47%) (40%, 55%)	400 (62%) (59%, 66%)	60 (73%) (62%, 82%)	66 (90%) (81%, 96%)
Missing	4	1	0	0	2	0	1
Symptoms in those seropositive							
Asymptomatic	21 (91%) (72%, 99%)	134 (88%) (81%, 92%)	57 (70%) (59%, 80%)	92 (100%) (96%, 100%)	362 (91%) (87%, 93%)	60 (100%) (94%, 100%)	65 (98%) (92%, 100%)
Symptomatic	2 (8.7%) (1.1%, 28%)	19 (12%) (7.6%, 19%)	24 (30%) (20%, 41%)	0 (0%) (0.00%, 3.9%)	38 (9.5%) (6.8%, 13%)	0 (0%) (0.00%, 6.0%)	1 (1.5%) (0.04%, 8.2%)
Symptoms in those seronegative							
Asymptomatic	24 (100%) (86%, 100%)	61 (81%) (71%, 89%)	12 (46%) (27%, 67%)	101 (99%) (95%, 100%)	223 (93%) (89%, 96%)	22 (100%) (85%, 100%)	7 (100%) (59%, 100%)

	PeriCOVID Malawi			PeriCOVID Uganda		COMAC Uganda	
Characteristic	Wave 2, N = 51 N (%) (95% CI)	Wave 3, N = 229 N (%) (95% CI)	Wave 4, N = 107 N (%) (95% CI)	Wave 1, N = 194 N (%) (95% CI)	Wave 2, N = 642 N (%) (95% CI)	Wave 2, N = 82 N (%) (95% CI)	Wave 3, N = 74 N (%) (95% CI)
Symptomatic	0 (0%) (0.00%, 14%)	14 (19%) (11%, 29%)	14 (54%) (33%, 73%)	1 (1.0%) (0.02%, 5.3%)	17 (7.1%) (4.2%, 11%)		

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288 Table 4 – Cord blood serology

	PeriCOVID Malawi			PeriCOVID Uganda		COMAC Uganda	
Characteristic	Wave 2, N = 52 N (%) (95% CI)	Wave 3, N = 230 N (%) (95% CI)	Wave 4, N = 111 N (%) (95% CI)	Wave 1, N = 195 N (%) (95% CI)	Wave 2, N = 642 N (%) (95% CI)	Wave 2, N = 82 N (%) (95% CI)	Wave 3, N = 74 N (%) (95% CI)
Concordance of maternal and cord blood samples							
Concordant	35 (80%) (65%, 90%)	194 (92%) (88%, 96%)	101 (96%) (91%, 99%)	79 (82%) (73%, 89%)	271 (72%) (67%, 76%)	81 (99%) (93%, 100%)	68 (96%) (88%, 99%)
Discordant	9 (20%) (9.8%, 35%)	16 (7.6%) (4.4%, 12%)	4 (3.8%) (1.0%, 9.5%)	17 (18%) (11%, 27%)	106 (28%) (24%, 33%)	1 (1.2%) (0.03%, 6.6%)	3 (4.2%) (0.88%, 12%)
Missing	8	20	6	99	265	0	3
Cord blood results with positive maternal blood result							
Negative	8 (40%) (19%, 64%)	14 (10%) (5.7%, 17%)	3 (3.8%) (0.78%, 11%)	8 (18%) (8.2%, 33%)	38 (16%) (12%, 21%)	0 (0%) (0.00%, 6.0%)	3 (4.7%) (0.98%, 13%)
Positive	12 (60%) (36%, 81%)	123 (90%) (83%, 94%)	77 (96%) (89%, 99%)	36 (82%) (67%, 92%)	198 (84%) (79%, 88%)	60 (100%) (94%, 100%)	61 (95%) (87%, 99%)
Missing	3	15	3	48	165	0	2
Cord blood result with negative maternal blood result							
Negative	23 (96%) (79%, 100%)	71 (97%) (90%, 100%)	24 (96%) (80%, 100%)	43 (83%) (70%, 92%)	73 (52%) (43%, 60%)	21 (95%) (77%, 100%)	7 (100%) (59%, 100%)

	PeriCOVID Malawi			PeriCOVID Uganda		COMAC Uganda	
Characteristic	Wave 2, N = 52 N (%) (95% CI)	Wave 3, N = 230 N (%) (95% CI)	Wave 4, N = 111 N (%) (95% CI)	Wave 1, N = 195 N (%) (95% CI)	Wave 2, N = 642 N (%) (95% CI)	Wave 2, N = 82 N (%) (95% CI)	Wave 3, N = 74 N (%) (95% CI)
Positive	1 (4.2%) (0.11%, 21%)	2 (2.7%) (0.33%, 9.5%)	1 (4.0%) (0.10%, 20%)	8 (15%) (6.9%, 28%)	68 (48%) (40%, 57%)	1 (4.5%) (0.12%, 23%)	0 (0%) (0.00%, 41%)
Missing	1	4	3	52*	98		

* 1 cord blood sample in PeriCOVID Uganda was insufficient for analysis

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SARS-CoV-2 infection in pregnancy and key adverse pregnancy and neonatal outcomes

1220/1224 mothers had serology results at enrolment available for analysis and were included in the analysis of Sars-CoV-2 infection and pregnancy outcomes. 79/1220 mothers experienced at least one adverse pregnancy outcome (maternal death N=4, abortion N=4, premature labour N=52, and stillbirth N=26) and there were 46 infant deaths (Supp Table 4 and 5). Of the 79 adverse pregnancy outcomes, 34 (43%) mothers were sero-negative, and 45 (57%) mothers were sero-positive, compared to 435 (38%) mothers who were sero-negative and 706 (62%) mothers who were sero-positive with no adverse pregnancy outcomes. There was no difference in pregnancy outcomes due to sero-positive SARS-CoV-2 status (Table 5). Due to the small numbers of outcomes, the impact of Sars-CoV-2 infection on maternal death could not be modelled. There was also no difference in outcomes due to SARS-CoV-2 infection with and without symptoms in the 14 days (periCOVID Uganda and Malawi) -28 days (COMAC) prior to enrolment (Supp Table 6).

305

306 Table 5– Impact of infection (seropositivity) on key pregnancy and neonatal outcomes in Pericovid Malawi and Pericovid Uganda

	Number included in model	Number of events	Relative Risk	95% Confidence Interval
Maternal death				
Sero-Negative		1	—	—
Sero-Positive		3	—	—
Infant death				
Sero-Negative	471	20	—	—
Sero-Positive	752	26	0.81	0.46, 1.46
Premature labour				
Sero-Negative	476	20	—	—
Sero-Positive	758	32	0.97	0.57, 1.71
Still birth				
Sero-Negative	473	15	—	—
Sero-Positive	752	11	0.48	0.22, 1.03
Abortion				
Sero-Negative		0	—	—
Sero-Positive		4	—	—
Combined adverse pregnancy outcome				
Sero-Negative	463	34	—	—
Sero-Positive	742	45	0.81	0.53, 1.26
Low birth weight				
Sero-Negative	474	22	—	—
Sero-Positive	753	39	0.98	0.60, 1.65
NICU admission				
Sero-Negative	471	63	—	—

Sero-Positive	753	107	0.96	0.73, 1.28
Combined adverse neonatal outcome				
Sero-Negative	476	77	—	—
Sero-Positive	758	120	0.92	0.71, 1.19

307 Models are adjusted for country

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There were 197 adverse infant outcomes (at least one of: infant/neonatal death, prematurity, low birth weight, NICU admission and birth asphyxia). There were 46 infant deaths, 26 (57%) from sero-positive and 20 (43%) from sero-negative women (Supp Table 5). There was no difference in risk of infant death due to SARS-CoV-2 serology status in the mother (Table 5). 77 (39%) infants with adverse outcomes were born to women who were sero-negative and 120 (61%) sero-positive. The relative risk was 0.92 (95% CI 0.71, 1.19), providing no evidence of a difference in the risk of at least one adverse neonatal outcome due to serological status of the mother. There was also no evidence of a difference in neonatal outcome due to positive serological status with and without symptoms in the mother (Supp Tables 4 and 5).

Placental transfer of SARS-CoV-2 antibodies in those with prior infection and/or vaccination

In PeriCOVID Uganda, 208/503 mother-infant pairs had anti-S IgG results available for analysis, corresponding to 27 and 181 during the first and second waves respectively. There was no difference between the maternal and cord blood anti-S IgG GMCs during the first wave. The GMR in the second wave was 1.7 (1.3, 2.3), indicating that anti-S IgG was higher in the cord blood than the maternal blood at enrolment. In comparison, in COMAC Uganda the GMR indicated no difference between the maternal and cord blood anti-S IgG for the 60 mother-infant pairs enrolled during the second wave, or for the 59 enrolled during the third wave (Supp Table 7). The rate of placental transfer of anti-S IgG is plotted in Fig 3.

Fig 3. Placental transfer of anti-s IgG in A) PeriCOVID Uganda and B) COMAC Uganda. Geometric mean ratios (GMRs) of anti-spike IgG for each mother-infant pair with results available for analysis on the Euroimmune anti-spike IgG assay. Boxplots of the GMRs show no evidence of a difference in placental transfer in different waves of the pandemic.

There were 39 mother-infant pairs enrolled in PeriCOVID Uganda during the first wave with anti-NCP results, and 194 during the second wave. For both waves, there was no evidence

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of a difference in the maternal and cord blood anti-NCP IgG results. In COMAC Uganda, 55 and 43 mother-infant pairs enrolled during the second and third waves, respectively, had anti-NCP IgG results. The GMR in the second wave was 1.8 (1.1, 2.9), and in the third wave was 2.7 (1.7, 4.4), indicating that anti-NCP IgG was higher in cord blood samples than in the maternal blood (Supp Table 7). The rate of placental transfer of anti-NCP IgG is plotted in Fig 3 . There was no clear evidence of a difference in placental transfer of both anti-S and anti-NCP IgG during different waves (Fig 3 and Fig S1).

Number of vaccinated women during pregnancy

A total of 29 participants across all sites reported prior vaccination to SARS-CoV-2: 7 from Malawi, 1 from periCOVID Uganda and 21 from COMAC. Monthly numbers of positive results in COMAC Uganda by vaccination status can be seen in Fig S2.

Discussion

This study describes the increasing prevalence of SARS CoV-2 infection across 2 countries and 5 hospital sites in East and southern Africa, and across several COVID-19 waves. This increase in prevalence coincided with waves of delta and omicron infection within the countries, respectively. Our data indicates that the majority of cases were asymptomatic and adds to existing evidence that suggests under-reporting of infection if based solely on confirmed cases by PCR(4, 5, 21).

The high prevalence of poor maternal and child health outcomes in sub-Saharan Africa, combined with the known impact of SARS-CoV-2 illness in pregnancy from existing studies

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outside of Africa (22-26), means that we need to better understand the direct effects of exposure to COVID-19 in pregnancy and outcome for the pregnant woman and her infant. The INTERCOVID study (27), which included 2 sites in West Africa (Nigeria and Ghana) showed that infection in pregnancy was associated with increased maternal and neonatal morbidity and mortality. The AFREHEALTH study of 1315 hospitalized pregnant and non-pregnant women with and without SARS-CoV-2 revealed an increased risk of ICU admission and in-hospital death amongst pregnant women with COVID-19 (28). Though we are unable to assess outcomes for symptomatic SARS-CoV-2 illness in our cohort due to low numbers our study does highlight that asymptomatic infection does not appear to be associated with death in the mother, or with worse neonatal outcomes in the first month of life. This is reassuring to parents and health care providers. Furthermore, in our study placental transfer of IgG increased during each subsequent wave. Previous studies earlier in the pandemic suggested reduced placental transfer of IgG in women with a positive SARS-CoV-2 RT-PCR (29, 30). More research is needed to better understand placental transfer with different SARS-CoV-2 variants.

Limitations

Our study is limited by differences in methodology across sites, with sampling performed at different time points in pregnancy, despite efforts to adhere to the UNITY protocol. The uniform collection of cord blood across all sites however enables comparison across sites and strengthens our results. In PeriCOVID Uganda, a strict period of lockdown over the summer of 2021 with a ban on public transport made it challenging for participants to attend hospital for delivery, leading to a lower number of cord blood samples than

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anticipated. Calls to participants by healthcare visitors were increased to ensure that women were aware that study staff were still working and could care for them during their delivery. We report our primary outcomes using the Wantai assay, but for placental antibody transfer, we report IgG using Euroimmune results. However, we identified cross-reactivity of antibodies against *Plasmodium falciparum* or other common cold coronaviruses (CCCs) as has been reported elsewhere in East Africa (21), meaning these results should be reviewed with caution. We had initially planned to use the Euroimmune anti-NCP assay to differentiate between infection and vaccination. However, the specificity of the assay precluded its use for this purpose. The Euroimmune anti-NCP assay has a lower sensitivity than other assays (31). Several studies have shown a low anti-NCP positivity after mild infections (32). As the pandemic progressed the chance of repeat SARS-CoV-2 infection increased, though these infections were generally milder (32). Anti-NCP antibodies in some studies have remained negative in individuals who were vaccinated against SARS-CoV-2 and who had an rt-PCR confirmed illness (32). A lower anti-NCP seropositivity later in the pandemic may therefore represent assay sensitivity and a lower anti-NCP immune response following mild or asymptomatic infection.

Conclusion

Data from Uganda and Malawi showed a seroprevalence of SARS-CoV-2 higher than the cases figures identified by other sources. In future pandemics and outbreaks, seroprevalence studies may be a more accurate measure in assessing the true prevalence of infection.

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References

1. Massinga Loembé M, Tshangela A, Salyer SJ, Varma JK, Ouma AEO, Nkengasong JN. COVID-19 in Africa: the spread and response. Nature Medicine. 2020;26(7):999-1003.

2. Abatan B, Agboghoroma O, Akemoke F, Antonio M, Awokola B, Bittaye M, et al. Intense and Mild First Epidemic Wave of Coronavirus Disease, The Gambia. Emerging infectious diseases. 2021;27(8):2064.

3. Martinez-Alvarez M, Jarde A, Usuf E, Brotherton H, Bittaye M, Samateh AL, et al. COVID-19 pandemic in west Africa. The lancet global health. 2020;8(5):e631-e2.

4. Adams J, MacKenzie MJ, Amegah AK, Ezeh A, Gadanya MA, Omigbodun A, et al. The conundrum of low COVID-19 mortality burden in sub-Saharan Africa: myth or reality? Global Health: Science and Practice. 2021;9(3):433-43.

5. Bamgboye EL, Omiye JA, Afolaranmi OJ, Davids MR, Tannor EK, Wadee S, et al. COVID-19 pandemic: is Africa different? Journal of the National Medical Association. 2021;113(3):324-35.

6. Norton A, Gozalo ADLH, De Colombi NF, Alobo M, Asego JM, Al-Rawni Z, et al. The remaining unknowns: a mixed methods study of the current and global health research priorities for COVID-19. BMJ Global Health. 2020;5(7):e003306.

7. Tsinda EK, Mmbando GS. Recent updates on the possible reasons for the low incidence and morbidity of COVID-19 cases in Africa. Bulletin of the National Research Centre. 2021;45(1):1-8.

8. Sié A, Hanefeld J, Chaponda M, Chico RM, LeDoare K, Mayaud P, et al. Congenital malformations in sub-Saharan Africa—warnings of a silent epidemic? The Lancet Infectious Diseases. 2021;21(5):594-6.

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9. Shah AV, Howell HB, Kazmi SH, Zaccario M, Sklamberg FE, Groth T, et al. Developmental screening of full-term infants at 16 to 18 months of age after in-utero exposure to maternal SARS-CoV-2 infection. Journal of Perinatology. 2023;43(5):659-63.

10. Edlow AG, Castro VM, Shook LL, Haneuse S, Kaimal AJ, Perlis RH. Sex-Specific Neurodevelopmental Outcomes Among Offspring of Mothers With SARS-CoV-2 Infection During Pregnancy. JAMA Network Open. 2023;6(3):e234415-e.

11. Emily RS, Erin O, Gargi Wable G, Kacey F, Fouzia F, Yalda A, et al. Adverse maternal, fetal, and newborn outcomes among pregnant women with SARS-CoV-2 infection: an individual participant data meta-analysis. BMJ Global Health. 2023;8(1):e009495.

12. Wilson SE, Deeks SL, Hatchette TF, Crowcroft NS. The role of seroepidemiology in the comprehensive surveillance of vaccine-preventable diseases. Cmaj. 2012;184(1):E70-E6.

13. Lai C-C, Wang J-H, Hsueh P-R. Population-based seroprevalence surveys of anti-SARS-CoV-2 antibody: An up-to-date review. International Journal of Infectious Diseases. 2020;101:314-22.

14. Chibwana MG, Jere KC, Kamn'gona R, Mandolo J, Katunga-Phiri V, Tembo D, et al. High SARS-CoV-2 seroprevalence in health care workers but relatively low numbers of deaths in urban Malawi. medrxiv. 2020.

15. Shaw JA, Meiring M, Cummins T, Chegou NN, Claassen C, Du Plessis N, et al. Higher SARS-CoV-2 seroprevalence in workers with lower socioeconomic status in Cape Town, South Africa. PLoS One. 2021;16(2):e0247852.

16. Uyoga S, Adetifa IM, Karanja HK, Nyagwange J, Tuju J, Wanjiku P, et al. Seroprevalence of anti–SARS-CoV-2 IgG antibodies in Kenyan blood donors. Science. 2021;371(6524):79-82.

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17. WHO. Generic protocol: a prospective cohort study investigating maternal, pregnancy and neonatal outcomes for women and neonates infected with SARS-CoV-2. Geneva World Health Organisation 2020

18. WHO. WHO COVID-19 Case definition 2020 [Available from: https://www.who.int/publications/i/item/WHO-2019-nCoV-Surveillance_Case_Definition-2020.2.

19. FIND DFA. FIND EVALUATION OF SARS-COV-2 ANTIBODY (AB) DETECTION TESTS 2021 [cited 2022 April 21st]. Available from: https://www.finddx.org/sarscov2-eval-antibody/.

20. Edouard Mathieu HR, Lucas Rodés-Guirao, Cameron Appel, Daniel Gavrilov, Charlie Giattino, Joe Hasell, Bobbie Macdonald, Saloni Dattani, Diana Beltekian, Esteban Ortiz-Ospina, and Max Roser. Coronavirus Pandemic (COVID-19) 2023 [Available from: https://ourworldindata.org/coronavirus#coronavirus-country-profiles.

21. Lewis HC, Ware H, Whelan M, Subissi L, Li Z, Ma X, et al. SARS-CoV-2 infection in Africa: a systematic review and meta-analysis of standardised seroprevalence studies, from January 2020 to December 2021. BMJ Global Health. 2022;7(8):e008793.

22. Zambrano LD, Ellington S, Strid P, Galang RR, Oduyebo T, Tong VT, et al. Update: Characteristics of Symptomatic Women of Reproductive Age with Laboratory-Confirmed SARS-CoV-2 Infection by Pregnancy Status - United States, January 22-October 3, 2020. MMWR Morb Mortal Wkly Rep. 2020;69(44):1641-7.

23. Badr DA, Mattern J, Carlin A, Cordier AG, Maillart E, El Hachem L, et al. Are clinical outcomes worse for pregnant women at ≥20 weeks' gestation infected with coronavirus disease 2019? A multicenter case-control study with propensity score matching. Am J Obstet Gynecol. 2020;223(5):764-8.

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492 24. Allotey J, Stallings E, Bonet M, Yap M, Chatterjee S, Kew T, et al. Clinical
493 manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019
494 in pregnancy: living systematic review and meta-analysis. Bmj. 2020;370:m3320.
495 25. Budhram S, Vannevel V, Botha T, Chauke L, Bhoora S, Balie GM, et al. Maternal
496 characteristics and pregnancy outcomes of hospitalized pregnant women with SARS-CoV-2
497 infection in South Africa: An International Network of Obstetric Survey Systems-based
498 cohort study. Int J Gynaecol Obstet. 2021;155(3):455-65.
499 26. De Waard L, Langenegger E, Erasmus K, Van der Merwe T, Olivier SE, Du Toit N,
500 et al. Maternal and neonatal outcomes of COVID-19 in a high-risk pregnant cohort with and
501 without HIV. S Afr Med J. 2021;111(12):1174-80.
502 27. Villar J, Ariff S, Gunier RB, Thiruvengadam R, Rauch S, Kholin A, et al. Maternal
503 and Neonatal Morbidity and Mortality Among Pregnant Women With and Without COVID-
504 19 Infection: The INTERCOVID Multinational Cohort Study. JAMA Pediatrics.
505 2021;175(8):817-26.
506 28. Nachega JB, Sam-Agudu NA, Machekano RN, Rosenthal PJ, Schell S, de Waard L, et
507 al. Severe Acute Respiratory Syndrome Coronavirus 2 Infection and Pregnancy in Sub-
508 Saharan Africa: A 6-Country Retrospective Cohort Analysis. Clinical Infectious Diseases.
509 2022;75(11):1950-61.
510 29. Rubio R, Aguilar R, Bustamante M, Muñoz E, Vázquez-Santiago M, Santano R, et al.
511 Maternal and neonatal immune response to SARS-CoV-2, IgG transplacental transfer and
512 cytokine profile. Front Immunol. 2022;13:999136.
513 30. Edlow AG, Li JZ, Collier A-rY, Atyeo C, James KE, Boatin AA, et al. Assessment of
514 Maternal and Neonatal SARS-CoV-2 Viral Load, Transplacental Antibody Transfer, and
515 Placental Pathology in Pregnancies During the COVID-19 Pandemic. JAMA Network Open.
516 2020;3(12):e2030455-e.

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517 31. Otter A. Personal Communication 2023

518 32. Follmann D, Janes HE, Buhule OD, Zhou H, Girard B, Marks K, et al.

519 Antinucleocapsid Antibodies After SARS-CoV-2 Infection in the Blinded Phase of the

520 Randomized, Placebo-Controlled mRNA-1273 COVID-19 Vaccine Efficacy Clinical Trial.

521 Ann Intern Med. 2022;175(9):1258-65.

522

Supplementary Information

S1 Fig. Placental transfer of anti-n IgG in A) PeriCOVID Uganda and B) COMAC Uganda. Geometric mean ratios (GMRs) of anti-nucelocapsid IgG for each mother-infant pair with results available for analysis on the Euroimmune anti-nucleocapsid IgG assay. Boxplots of the GMRs show no evidence of a difference in placental transfer in different waves of the pandemic

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S2 Fig. Monthly number of positives in COMAC Uganda, by vaccination status The monthly number of women in COMAC Uganda who were seropositive at enrolment on each of the Wantai (top panel), Euroimmune anti-S (middle panel) and Euroimmune anti-N (bottom panel) assays, coloured by vaccination status. Green shows those who were unvaccinated at enrolment, blue shows the small proportion who were vaccinated, and red shows those whose vaccination status was unknown at enrolment. The majority were unvaccinated.

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S1 Table. Wantai Assay Specificity.

	Wantai positive (n)	Wantai negative (n)	Specificity (%)
2019 samples, malaria positive (n=74)	1	73	98.65
2019 samples, malaria negative (n=100)	1	99	99

S2 Table. Dates used to define COVID-19 waves in Malawi and Uganda

Country	Wave 1	Wave 2	Wave 3	Wave 4
Uganda	26/03/2020 – 21/03/2021	22/03/2021 – 30/11/2021	01/12/2021 – 30/04/2022	
Malawi	07/04/2020 – 16/11/2020	17/11/2020 – 15/05/2021	16/05/2021 – 11/11/2021	12/11/2021 – 20/04/2022

S3 Table – Maternal PCR tests by study site and wave

	PeriCOVID Malawi				PeriCOVID Uganda		
Characteristic	**Overall, N = 73 (95% CI)[12]**	**Wave 2, N = 2 (95% CI)[12]**	**Wave 3, N = 31 (95% CI)[12]**	**Wave 4, N = 40 (95% CI)[12]**	**Overall, N = 836 (95% CI)[12]**	**Wave 1, N = 194 (95% CI)[12]**	**Wave 2, N = 642 (95% CI)[12]**
PCR result							
Negative	24 (33%) (22%, 45%)	1 (50%) (1.3%, 99%)	11 (35%) (19%, 55%)	12 (30%) (17%, 47%)	817 (98%) (96%, 99%)	194 (100%) (98%, 100%)	623 (97%) (95%, 98%)
Positive	49 (67%) (55%, 78%)	1 (50%) (1.3%, 99%)	20 (65%) (45%, 81%)	28 (70%) (53%, 83%)	19 (2.3%) (1.4%, 3.5%)	0 (0%) (0.00%, 1.9%)	19 (3.0%) (1.8%, 4.6%)
Symptoms in those with positive PCR							
Asymptomatic	2 (4.1%) (0.50%, 14%)	0 (0%) (0.00%, 98%)	0 (0%) (0.00%, 17%)	2 (7.1%) (0.88%, 24%)	13 (68%) (43%, 87%)	NA	13 (68%) (43%, 87%)
Symptomatic	47 (96%) (86%, 100%)	1 (100%) (2.5%, 100%)	20 (100%) (83%, 100%)	26 (93%) (76%, 99%)	6 (32%) (13%, 57%)	NA	6 (32%) (13%, 57%)

Symptoms are those defined as probable infection according to WHO criteria (18)

S4 Table – Adverse pregnancy outcomes in mothers enrolled in PeriCOVID Malawi and PeriCOVID Uganda

	All Enrolled	Wantai Overall	Wantai Serostatus	
Characteristic	**N = 1,224[1]**	**N = 1,220[1]**	**Negative, N = 469[2]**	**Positive, N = 751[2]**
Maternal death	4 (0.3%)	4 (0.3%)	1 (25%)	3 (75%)
Unknown	27	25	9	16
Abortion	4 (0.3%)	4 (0.3%)	0 (0%)	4 (100%)
Unknown	25	24	9	15
Premature labour	52 (4.3%)	52 (4.3%)	20 (38%)	32 (62%)
Unknown	1			
Stillbirth	26 (2.1%)	26 (2.1%)	15 (58%)	11 (42%)
At least one adverse pregnancy outcome	79 (6.5%)	79 (6.5%)	34 (43%)	45 (57%)
No adverse pregnancy outcome	1,145 (94%)	1,141 (94%)	435 (38%)	706 (62%)
Livebirth	1,198 (98%)	1,194 (98%)	454 (38%)	740 (62%)

[1] Column percentages are presented for the overall number of women experiencing each outcome

[2] Row percentages are presented for the number of seropositive and seronegative women experiencing each outcome

S5 Table - Adverse neonatal outcomes in infants born in PeriCOVID Malawi and PeriCOVID Uganda

	All infants	Wantai Overall	Maternal Wantai Serostatus	
Characteristic	**N = 1,230[1]**	**N = 1,227[1]**	**Negative, N = 474[2]**	**Positive, N = 753[2]**
Infant death	46 (3.8%)	46 (3.8%)	20 (43%)	26 (57%)
Unknown	5	4	3	1
Prematurity	58 (4.7%)	58 (4.7%)	22 (38%)	36 (62%)
Unknown	1			
Low birth weight	61 (5.0%)	61 (5.0%)	22 (36%)	39 (64%)
Unknown	1			
Admitted to NICU	170 (14%)	170 (14%)	63 (37%)	107 (63%)
Unknown	4	3	3	0
Birth asphyxia	53 (4.3%)	53 (4.3%)	20 (38%)	33 (62%)
Unknown	1			
At least one adverse neonatal outcome	197 (16%)	197 (16%)	77 (39%)	120 (61%)
No adverse neonatal/infant outcome	1,033 (84%)	1,030 (84%)	397 (39%)	633 (61%)

[1] Column percentages are presented for the overall number of infants experiencing each outcome

[2] Row percentages are presented for the number of infants born to seropositive and seronegative women with each outcome

551 **S6 Table - Impact of infection (seropositive and WHO probable) on key pregnancy and neonatal outcomes**

	Number included in model	Number of events	Relative Risk	95% Confidence Interval
Maternal death				
Infection status		4		
sero-negative		1	—	—
sero-positive and not WHO probable		0	—	—
sero-positive and WHO probable		3	—	—
Infant death				
Infection status	1,223	46		
sero-negative	471	20	—	—
sero-positive and not WHO probable	667	24	0.85	0.47, 1.53
sero-positive and WHO probable	85	2	—	—
Premature labour				
Infection status	1,234	52		
Sero- negative	476	20	—	—
Sero- positive and not WHO probable	672	27	0.94	0.53, 1.67
Sero- positive and WHO probable	86	5	1.24	0.42, 3.00
Still birth				
Infection status	1,225	26		
Sero- negative	473	15	—	—
Sero- positive and not WHO probable	667	10	0.48	0.21, 1.05
Sero- positive and WHO probable	85	1	—	—
Abortion				
Infection status		4		
Sero- negative		0	—	—
Sero- positive and not WHO probable		4	—	—
Sero- positive and WHO probable		0	—	—

Combined adverse pregnancy outcome				
Infection status	1,205	79		
Sero- negative	463	34	—	—
Sero- positive and not WHO probable	659	36	0.74	0.47, 1.16
Sero- positive and WHO probable	83	9	1.39	0.64, 2.68
Low birth weight				
Infection status	1,227	61		
Sero- negative	474	22	—	—
Sero- positive and not WHO probable	668	33	0.99	0.59, 1.67
Sero- positive and WHO probable	85	6	0.98	0.37, 2.17
NICU admission				
Infection status	1,224	170		
Sero- negative	471	63	—	—
Sero- positive and not WHO probable	668	90	0.95	0.72, 1.28
Sero- positive and WHO probable	85	17	1.03	0.62, 1.60
Combined adverse neonatal outcome				
Infection status	1,234	197		
Sero- negative	476	77	—	—
Sero- positive and not WHO probable	672	102	0.91	0.70, 1.19
Sero- positive and WHO probable	86	18	0.98	0.60, 1.50

552 Models are adjusted for country

S7 Table - Placental Transfer

		PeriCOVID Uganda		COMAC Uganda	
		Wave 1	Wave 2	Wave 2	Wave 3
Anti-S	Infant, GMC (95% CI)	78.8 (46.4, 133.7) [N = 27]	127.6 (100.6, 161.8) [N = 181]	152 (100.3, 230.3) [N = 60]	65.2 (47.1, 90.4) [N = 59]
	Mother, GMC (95% CI)	51.7 (34.6, 77.3) [N = 27]	74.7 (59.3, 94.2) [N = 181]	96.1 (65.9, 140.1) [N = 60]	98.5 (68.6, 141.5) [N = 59]
	Placental transfer, GMR (95% CI)	1.5 (0.9, 2.5) [N = 27]	1.7 (1.3, 2.3) [N = 181]	1.6 (0.8, 3) [N = 60]	0.7 (0.4, 1) [N = 59]
Anti-N	Infant, GMC (95% CI)	57.1 (38, 85.8) [N = 39]	133.7 (106.5, 167.8) [N = 194]	155.9 (102.1, 237.9) [N = 55]	172 (103, 287.2) [N = 43]
	Mother, GMC (95% CI)	57.6 (41.5, 79.9) [N = 39]	146.2 (114.4, 186.8) [N = 194]	87.6 (54.9, 139.8) [N = 55]	62.9 (35.5, 111.4) [N = 43]
	Placental transfer, GMR (95% CI)	1 (0.6, 1.6) [N = 39]	0.9 (0.7, 1.3) [N = 194]	1.8 (1.1, 2.9) [N = 55]	2.7 (1.7, 4.4) [N = 43]

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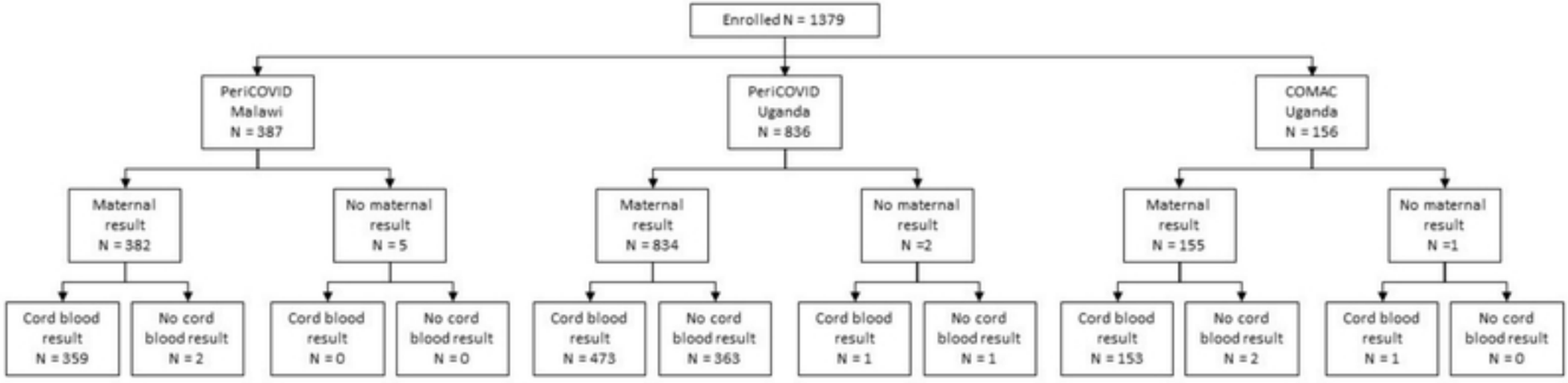
Figure 1 – Study flow chart



Study flow chart to show the number of women enrolled by study, with maternal serology available at enrolment (maternal result) and the infants with cord blood serology (cord blood result) available

Figure 1

Figure 2 – Monthly sero-positivity by country in pregnant women

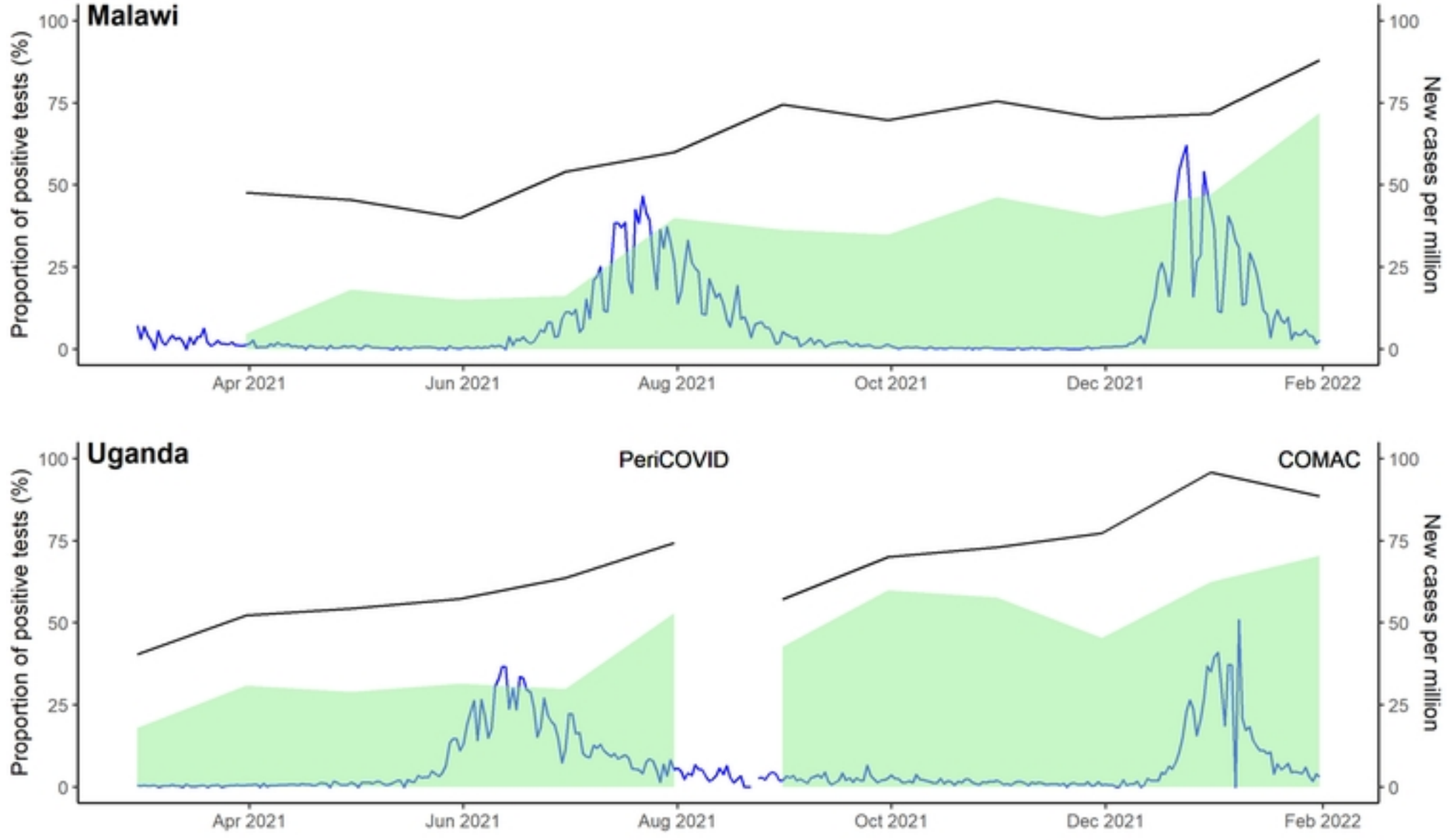
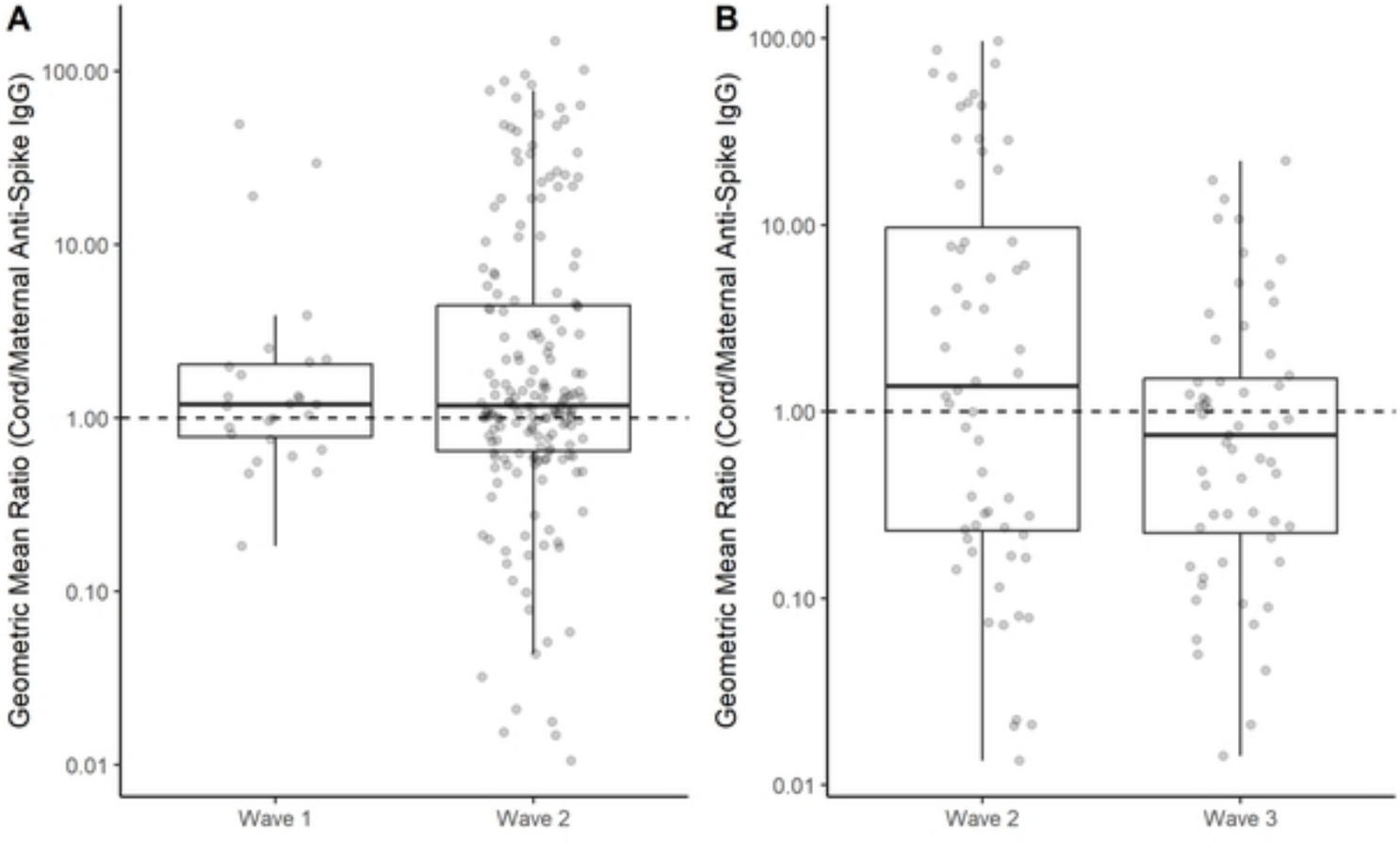


Figure 2

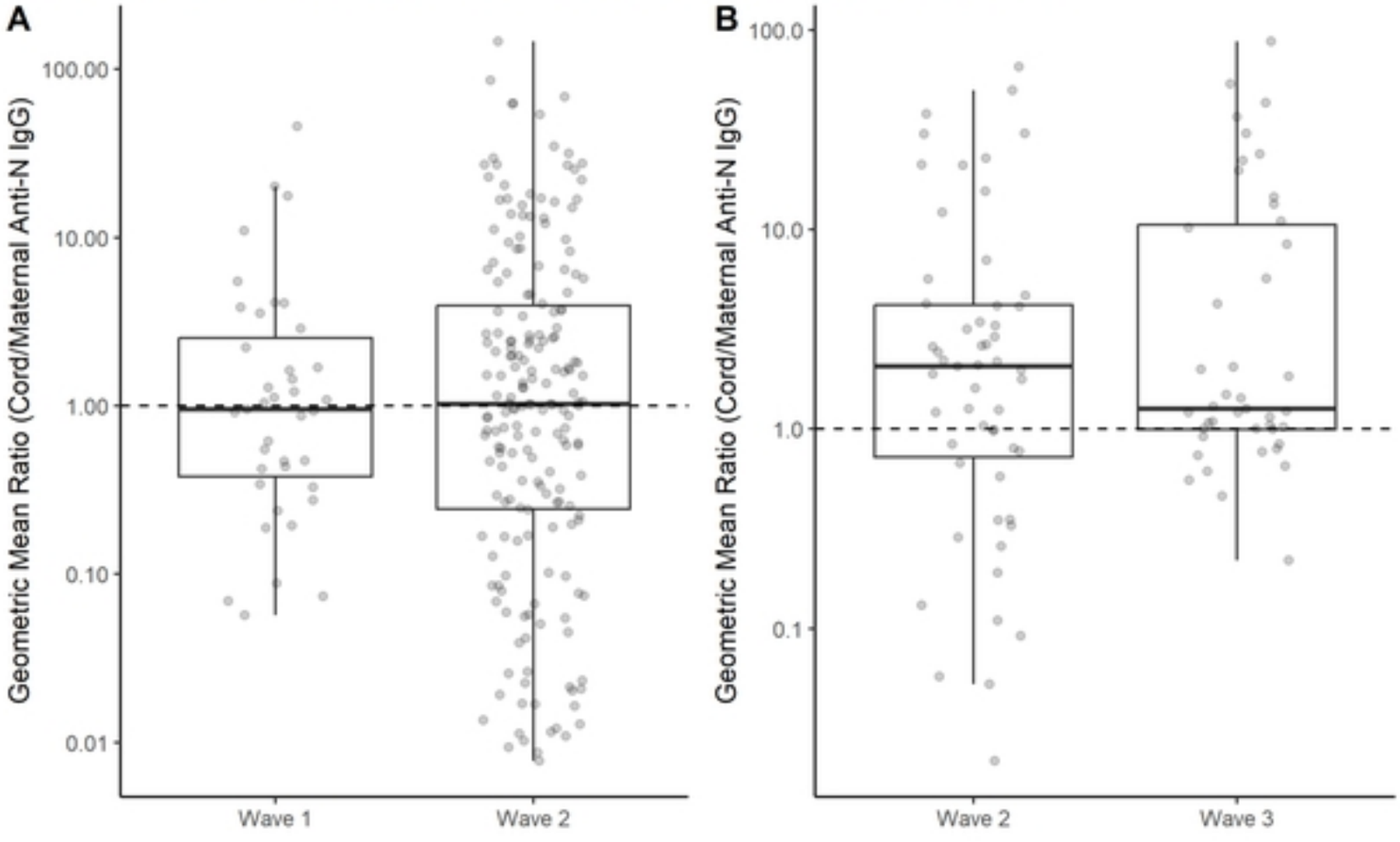
Figure 3 – Placental transfer of anti-s IgG in A) PeriCOVID Uganda and B) COMAC Uganda



Geometric mean ratios (GMRs) of anti-spike IgG for each mother-infant pair with results available for analysis on the Euroimmune anti-spike IgG assay. Boxplots of the GMRs show no evidence of a difference in placental transfer in different waves of the pandemic.

Figure 3

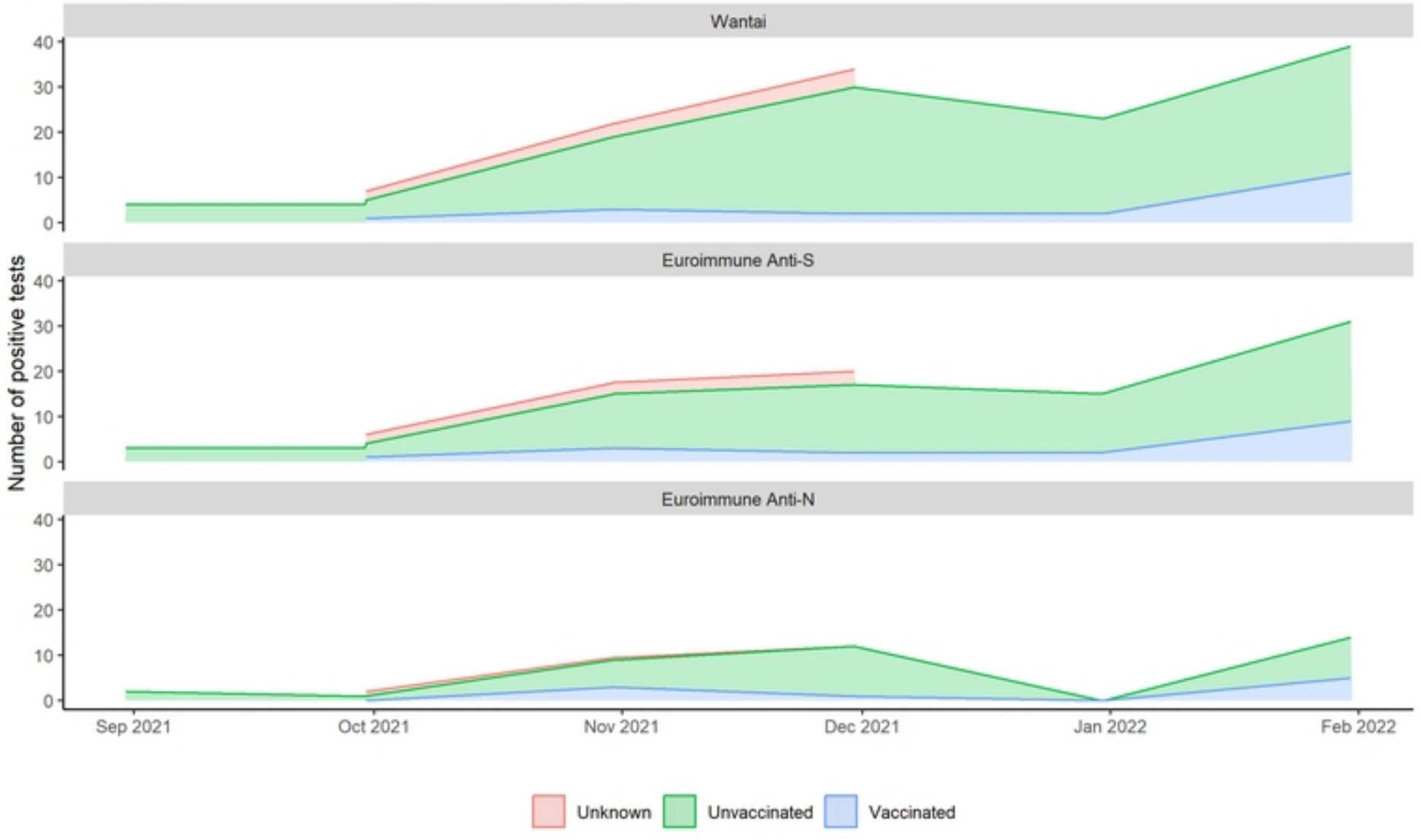
Figure S1 – Placental transfer of anti-n IgG in A) PeriCOVID Uganda and B) COMAC Uganda



Geometric mean ratios (GMRs) of anti-nucelocapsid IgG for each mother-infant pair with results available for analysis on the Euroimmune anti-nucleocapsid IgG assay. Boxplots of the GMRs show no evidence of a difference in placental transfer in different waves of the pandemic

Supp Figure 1

Figure S2 - Monthly number of positives in COMAC Uganda, by vaccination status



The monthly number of women in COMAC Uganda who were seropositive at enrolment on each of the Wantai (top panel), Euroimmune anti-S (middle panel) and Euroimmune anti-N (bottom panel) assays, coloured by vaccination status. Green shows those who were unvaccinated at enrolment, blue shows the small proportion who were vaccinated, and red shows those whose vaccination status was unknown at enrolment. The majority were unvaccinated.

Supp Figure 2