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Prevalence and impact of SARS-CoV-2 infection on maternal and infant health in African populations: protocol of a multi-centre prospective cohort study (MA-CoV project)

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ABSTRACT

Introduction. Pregnant women are currently considered a vulnerable population to SARS-CoV-2 infection, with increased risk of severe COVID-19, pre-term birth and maternal mortality. There is, however, a paucity of data on the burden of maternal SARS-CoV-2 infection in sub-Saharan countries. The objective of this study is to determine the prevalence and health effects of maternal SARS-CoV-2 infection in selected sites from Gabon and Mozambique.

Methods and analysis. MA-CoV (MAternal CoVid) is an observational, multicenter, prospective cohort study where 1000 pregnant women (500 per country) will be enrolled at the antenatal clinic visits. Participants will undergo monthly follow-up at each antenatal care visit, delivery and post-partum visit. The primary study outcome is the prevalence of SARS-CoV-2 infection during pregnancy. The clinical presentation of COVID-19 in pregnancy will also be characterized, and incidence of infection during pregnancy will be evaluated, as well as the risk factors of maternal and neonatal morbidity and mortality associated with SARS-CoV-2 infection and the risk of mother-to-child transmission of SARS-CoV-2. SARS-CoV-2 infection screening will be performed through polymerase chain reaction (PCR) diagnosis.

Ethics and dissemination. The protocol was reviewed and approved by the institutional and national ethics committees of Gabon and Mozambique and the Hospital Clinic of Barcelona. Project results will be presented to all stakeholders and published in open-access journals.

Registration number. NCT05303168.

Strengths and limitations

- MA-CoV will provide important information of SARS-CoV-2 infection in pregnancy from a large number of participants in settings where malaria and HIV are endemic, allowing to better understand co-infection.
- The inclusion of participants from two different sub-Saharan countries will provide information on the differences in the distribution of SARS-CoV-2 infection.
- The COVID-19 pandemic scenario presents several barriers and challenges not only due to the disease itself but also due to the implementation of containment measures such as quarantine, social distancing and community containment. This may affect antenatal care attendance, as well as hinder the implementation of the study.
- As COVID-19 vaccination of study participants may limit our capacity to estimate the prevalence of the infection through SARS-CoV-2 serology, we will assess the prevalence of antibodies against the SARS-CoV-2 nucleocapsid protein, which reflects past SARS-CoV-2 infection. In addition, this will allow us to estimate the effectiveness of SARS-CoV-2 vaccination in this cohort.

Keywords: SARS-CoV-2, sub-Saharan Africa, pregnancy

INTRODUCTION

As of July 2022, more than nine million COVID-19 cases and 170,000 related deaths have been reported in the World Health Organization (WHO) African region, while only 21% of the African population has been fully vaccinated.[1] However, the real burden of SARS-CoV-2 in Africa is probably still unknown and underestimated.

Pregnant women are at increased susceptibility of SARS-CoV-2 infection, particularly those with co-morbidities such as preeclampsia and gestational diabetes mellitus.[2] This may be explained by the pregnancy-induced changes, which include a decreased lung volume, an increased risk for thromboembolic disease and immunological changes in order to allow for the growth of a semi-allogenic fetus.[3] Effects of SARS-CoV-2 infection on maternal and neonatal health include increased risk of admission to intensive care and need of mechanical ventilation, induced abortion, c-section, pre-term birth, foetal growth restriction, post-partum hemorrhage and maternal mortality. [4-6]

Besides, mother to child transmission of SARS-CoV-2 is possible intrauterine, intrapartum, and at the postpartum period.[7] Several studies have reported the detection of SARS-CoV-2 in the fetal side of the placenta, indicating transplacental foetal infection.[7] Of note, most of the evidence of these effect has been gathered in high income countries.

In Sub-Saharan Africa (SSA), SARS-CoV-2 overlaps geographically with endemic infectious diseases such as the Human Immunodeficiency Virus (HIV) and malaria in a context of low SARS-CoV-2 vaccination coverage. For instance, co-infection with SARS-CoV-2 and malaria in pregnant women, might have deleterious effects in the foetal development, considering the reported inflammatory and histologic changes at the placental level found in both infections.[8 9] Additionally, there is evidence that immunosuppressed HIV-infected individuals are at increased risk of severe COVID-19 and death than non-infected individuals.[10 11] Importantly, the burden of HIV infection is concentrated in SSA.[12]

The information on the burden of SARS-CoV-2 infection in pregnancy in SSA countries is very limited. As to date, most of studies have been carried out in high income countries, neglecting the particular characteristics of SARS-CoV-2 infection in pregnancy in low-middle income countries. In this context, the present study was developed leveraging on an ongoing clinical trial of malaria prevention among HIV-infected pregnant women.[13]

Study aims and hypotheses

The primary objective of the MA-CoV (Maternal CoVid) study is to determine the prevalence and incidence of SARS-CoV-2 infection during pregnancy. Secondary objectives include to describe the effects of maternal SARS-CoV-2 infection on pregnancy and perinatal outcomes, to characterize the clinical features of COVID-19 disease in pregnancy, and to assess the potential vertical transmission and through breastfeeding of SARS-CoV-2 from infected mothers to their offspring. The main study hypotheses are: (1) SARS-CoV-2 infection during pregnancy may influence maternal and perinatal outcomes, (2) SARS-CoV-2 clinical manifestations may be different in pregnant women compared to non-pregnant adults, and (3) SARS-CoV-2 can be transmitted from mother to child prenatally and postnatally.

METHODS AND ANALYSIS

MA-CoV is an observational, multicenter prospective cohort study.

Study settings

The study will be carried out in Libreville and Lambaréné (Gabon), and in Manhiça (Mozambique). SARS-CoV-2 reported cases ranged from 48000 in Gabon to 228,000 in Mozambique as per July 2022.[14] Additionally, HIV prevalence among pregnant women ranges from 6% in study sites of Gabon to 29% in study sites of Mozambique.[15] [16] Malaria epidemiological indicators and SARS-CoV-2 and HIV prevalence in pregnancy in study sites are shown in table 1.

Table 1. SARS-CoV-2, malaria and HIV epidemiology in study countries

Country	Site	SARS-CoV-2 reported cases (country-level)	*P.falciparum* infection prevalence in women at delivery†	HIV prevalence in pregnant women
Mozambique	Manhiça	228,000[1]	6%	29%[15]
Gabon	Lambaréné	48,000[1]	11%	6%[16]
	Libreville		NI	6%[16]

†Data from 2010-2012 in women receiving either two IPTp doses of mefloquine or SP (Tuikue-Ndam et al, unpublished). NI: No information; MTCT: mother-to-child transmission

Study population

All pregnant women attending the study antenatal care (ANC) services will be screened for participation in the study. Inclusion criteria are: (1) permanent resident in the study area and (2) willing to deliver in the study maternity wards.

Informed consent and recruitment

All participants will receive information about study procedures. A signed informed consent form (or thumb-printed with a witness if the woman is illiterate) will be obtained before any study procedures are carried out by study nurses in each site. The informed consent will cover the woman and the new born infant. The study's informed consent is available as Supplemental Material 1. If the participant is under the legal age of maturity, she will sign the assent form and her legal guardian will sign the informed consent according to national ethics local policies.

After the study details are explained and informed consent is signed, a study identification card containing the individual study number and basic demographic information will be given to the participant in order to facilitate identification at all study contacts.

Follow-up and measurement of outcomes

At baseline, the woman's demographic and obstetric information will be recorded in study specific case report forms (CRFs).

Physical and clinical examination at enrolment

The physical examination of the woman will include the following assessments: weight, height, gestational age by bimanual palpation and measurement of middle-upper arm circumference (MUAC). Ultrasound will be performed to determine gestational age and confirm pregnancy viability at enrolment if possible. COVID-19 suggestive symptoms will be assessed, and should the woman present them, A nasopharyngeal swab will be collected for detection of SARS-CoV-2 viral RNA. Additionally, a nasopharyngeal swab will be collected in a sub-sample of 100 study participants regardless presence of COVID-19 symptoms for screening of SARS-CoV-2 infection.

Baseline biological samples

At enrolment, a venous blood sample (5 mL) will be collected for analysis of hemoglobin level, SARS-CoV-2 total antibodies, malaria polymerase chain reaction (PCR) (if the woman presents malaria-suggestive symptoms), and HIV viral load and CD4 cell count (if the woman is HIV-infected).

Antenatal follow-up

Participants will receive the standard ANC package of interventions, which includes intermittent preventive treatment (IPTp) of malaria, iron and folate supplementation, following national guidelines. During monthly ANC visits. COVID-19 suggestive symptoms will be assessed, and should the woman present them, a PCR to detect SARS-CoV-2 viral load will be performed.

End of pregnancy and post-partum period

At the end of pregnancy, 5 ml of maternal blood sample will be collected for analysis of antibodies (IgG and IgM) against SARS-CoV-2, malaria parasitaemia and HIV viral load (in case the woman is HIV-infected). Additionally, whenever possible, cord blood and placental tissue samples will be collected for SARS-CoV-2 serologic and PCR analysis, respectively.

Breastmilk samples (3 ml) will also be collected within the first three days after delivery (colostrum) and at the post-partum visit (approximately six weeks after the end of pregnancy), for detection of SARS-CoV-2 by PCR. In addition, a neonatal throat swab will be collected at birth for SARS-CoV-2 analysis by PCR in infants born to COVID-19 positive mothers. A summary of study procedures is displayed in Table 2.

Table 2. Study visits and procedures schedule

Study procedure	First ANC clinic visit	Routine ANC clinic visits	End of pregnancy	1 month after end of pregnancy	Unscheduled visits	Infant Assessment (birth and 1 month)
Inclusion/ Exclusion criteria check	X					
Written informed consent	X					
Demographics, socio-economic/ Medical history	X				X	
COVID-19 screening#	X	X			X	X
Record of medications/ Morbidity	X	X	X	X	X	
Physical examination/clinical	X		X		X	
Gestational age	X	X	X		X	
Temperature				X	X	X
Blood Pressure	X		X	X	X	
Weight	X	X		X	X	
Height	X					
MUAC	X			X		
Presence of proteins in urine	X					
CD4 count*	X					
HIV viral load*	X		X			
SARS-CoV-2 serology	X		X			
Malaria blood PCR	X					
Blood smear	†	†	X	X	†	
Haemoglobin test	X		X	X		
Peripheral venous blood (mother)	X		X			
Cord blood			X			
Placental biopsy			X			
Placental impression smears			X			
Breastmilk (SARS-CoV2)			X	X		

ANC: Antenatal care; MUAC: middle-upper circumference

In participants with suggestive symptoms/signs of COVID-19 (fever, cough, shortness of breath, sudden onset of anosmia, ageusia or dysgeusia), except in a sub-sample of 100 participants at enrolment among whom it will be performed regardless of presence of symptoms.

*Only in HIV-infected women

† Only in women passively reporting sick AND presenting with malaria related signs/symptoms (fever (≥37,5º C) or having history of fever in the past 24 hours, arthromyalgia or headache), as per national management guidelines

Infant assessment

A neonatal throat swab will be collected at birth for SARS-CoV-2 analysis by PCR in infants born to COVID-19 positive mothers. Should the neonate present with symptoms and/or signs suggestive of acute respiratory infection during the first month of life, another throat swab will be collected for SARS-CoV-2 testing by PCR.

Laboratory tests

Detection of SARS-CoV-2

A real-time polymerase chain reaction (RT-PCR) COVID-19 assay diagnostic test will be performed at the study laboratories for detection of SARS-CoV-2 viral RNA. Real-Time PCR technology utilizes polymerase chain reaction for the amplification of specific target sequences and target specific probes for the detection of the amplified RNA. The probes are labelled with fluorescent reporter and quencher dyes.

The Atellica IM Analyzer will be used for detection of total antibodies through a chemiluminescent immunoassay intended for the qualitative and semiquantitative detection of total antibodies (including IgG and IgM) to SARS-CoV-2 in serum and plasma.[17] The Atellica IM Analyzer is an automated antigen sandwich immunoassay using acridinium ester chemiluminescent technology, in which antigens are bridged by antibodies present in the sample [17].

Malaria parasitological and haematological determinations

In case of malaria suspicion, thick and thin blood smears will be collected and stained with Giemsa's stain and examined for *Plasmodium spp*. following standard procedures. Also, blood haemoglobin will be determined following local SOPs.

Detection of HIV and quantitative determination of viral load

In HIV-infected women, quantitative PCR HIV viral load will be determined from the venous blood samples drawn at enrolment and at delivery. HIV viral load will be determined from plasma cryopreserved at -80ºC using the devices in place *in the study sites (such as* COBAS® AMPLICOR, AmpliPrep [Roche Diagnostics] *or GeneXpert).*

Immunological determinations related to HIV status

In HIV-infected women, CD4+T cell count will be determined by flow cytometry after staining of whole blood with CD3, CD8 and CD4 fluorochrometolabelled antibodies and acquisition using FACSCalibur (BD Biosciences) and TruCOUNT tubes (Becton Dickinson, San Jose, CA; USA) *or MiniVIdas device*.

Placental samples analysis

A placental sample will be collected for malaria histological analysis. The biopsies will be immediately placed in 25 mL of 10% neutral buffered formalin and kept at 4ºC until processed and embedded in paraffin wax by standard techniques. Paraffin sections will be stained with haematoxylin and eosin, Giemsa's stain and the periodic acid-Schiff technique. Placental histology will include the examination of inflammatory signs (such as presence of neutrophils and monocytes) in the subchorial space and the umbilical cord connective tissue (funisitis) and analysis of intervillous fibrin deposition.[18]

Additionally, another placental sample will be collected for SARS-CoV-2 histopathological detection. The placental tissue will be placed in a sterile 150 ml bottle and kept in a -80ºC freezer until the sample is processed. Placental histology will include the examination of inflammatory signs (such as presence of neutrophils and monocytes) in the subchorial space and the umbilical cord connective tissue (funisitis) and analysis of intervillous fibrin deposition.[18]

Data management

All the data will be collected using paper CRFs during the study visits, from interviews and clinical observation or measures taken to participants. Results from the laboratory analyses performed in collected participant's biological samples will also be collected and entered in the CRFs.

Data from the study source document will be double entered into the study database using the OpenClinica open source software version 3.1.4 (Copyright OpenClinica LLC and collaborators, Waltham, MA, USA, www.OpenClinica.com) Subsequently, entered data will be systematically checked by Data Management team using error messages printed from validation programs and database listings. Quality control audits of all key safety and efficacy information in the database will be made prior to locking the database.

Study outcomes

The primary outcome of the study will be the prevalence of anti-SARS-CoV-2 N protein total antibodies at delivery. The secondary endpoints can be found in Table 3.

Table 3. Study endpoints

Primary endpoint	Prevalence of anti-SARS-CoV-2 N protein antibodies (Ig G and/or Ig M positive) against SARS-CoV-2 among pregnant women at delivery
Secondary endpoints	1. PCR-confirmed SARS-CoV-2 infection among pregnant women at recruitment 2. Incidence of SARS-CoV-2 infection during pregnancy 3. Maternal and neonatal morbidity and mortality due to SARS-CoV-2 infection during pregnancy 4. Pregnancy and perinatal adverse outcomes 5. Rate of vertical transmission of SARS-CoV-2 from infected mothers to their offspring, during the prenatal and perinatal period 6. CD4 cell counts and HIV viral load 7. Malaria parasitaemia at delivery (from maternal sample collected at delivery)

Sample size

Considering six months of enrolment and recruitment rates of participants in ongoing clinical trials in the two study sites, it was expected to include approximately 1000 women in the study. Assuming a 5% prevalence of SARS-CoV-2 of infection during pregnancy, this sample size would allow estimating the proportion of women with the infection with a 1.4% precision at the 95% confidence level.[19]

Statistical analysis

Infection by SARS-CoV-2 will be defined by presence of anti-SARS-CoV-2 nucleocapsid (N) protein antibodies (total IgG, IgG and/or IgM) or by a positive SARS-CoV-2 PCR. Women with baseline anti-SARS-CoV-2 N protein SARS-CoV-2 antibodies will be considered infected before study enrolment.

The socio-demographic characteristics of the study participants will be described using summary statistics. Continuous variables will be summarized using mean or median (depending on the distribution of the variable) and standard deviation or interquartile range. Categorical variables will be described using frequencies and percentages. Proportions for categorical variables will

be assessed using the chi-square test or Fisher's exact test where appropriate. The Student's t test or Wilcoxon rank-sum test will be used to compare means and medians, respectively, of continuous variables according to variable characteristics. Only records with information on the outcome of interest will be analyzed.

Incidences of all-cause hospital admissions and all-cause outpatient attendance during pregnancy will be analyzed using negative binomial regression and compared by SARS-CoV-2 infection status. The incidence of COVID-19 and clinical malaria episodes will be determined. The frequency of COVID-19 will be compared between HIV-infected and HIV-uninfected women using a negative binomial regression. The proportion of women with adverse pregnancy outcomes will be compared by SARS-CoV-2 infection status using a modified binomial regression. These analyses will be done unadjusted and adjusted by baseline significant variables (age, gestational age, gravidity, RPR, anaemia and literacy, study intervention) and clinically relevant factors depending on the outcome for control of confounding factors. Incidences of hospital admissions in the neonate will be also analyzed using negative binomial regression. Data analysis will be performed using Stata (Stata Corp).[20]

Patient and public involvement. Patients will not be directly involved in the design, conduct, reporting or dissemination plans of the study.

DISCUSSION

At the onset of the COVID-19 pandemic, the extent of the risks in pregnancy was uncertain. In this context, the MA-CoV study was conceived to address fundamental questions on the burden and effects of SARS-CoV-2 infection during pregnancy. MA-CoV is an international, prospective observational cohort study that plans to follow pregnant women living in study areas of Gabon and Mozambique, where malaria and HIV infections are endemic and the real burden of SARS-CoV-2 infection is still unknown. Participants will be followed at monthly ANC visits, until 6 weeks after end of pregnancy. Additionally, the presence of antibodies (IgG/IgM) against SARS-CoV-2 in blood samples will be determined. The clinical presentation of COVID-19 in pregnancy will also be characterized, and incidence of infection during pregnancy will be evaluated, as well as the risk factors of maternal and neonatal morbidity and mortality associated with SARS-CoV-2 infection and the risk of mother-to-child transmission of SARS-CoV-2. Recruitment is expected to finish in September 2022, while patient follow-up is expected to be completed in April 2023.

The effects of SARS-CoV-2 infection on maternal and neonatal health have been described mostly by studies performed in high income countries, and include increased risk of admission to intensive care, abortion, c-section, pre-term birth, foetal growth restriction, post-partum hemorrhage and maternal mortality. [4-6] A retrospective cohort study analyzing routine data that was performed in six SSA countries reported similar findings.[21] In addition, interaction of COVID-19 with other global epidemics such as HIV is particular relevant in the African region, given that it has the highest world incidence of HIV infection, being women of reproductive age at higher risk.[22] Recent studies have shown that HIV infection is associated with a significant increased risk of contracting SARS-CoV-2. In addition, immunosuppressed HIV-infected individuals have been shown to have higher incidence of severe COVID-19 and death than non-HIV-infected individuals. [10 11 23] Importantly, the study performed in six countries of SSA found that pregnant women with HIV had an increased risk of admission to intensive care.[21]

Pregnant women still face disproportionate inequalities in access to and quality health care. The most essential maternal and reproductive health interventions do not reach yet the poorest and most vulnerable women, girls and children in the developing world. This results in marked poor

understanding of the particular characteristics and health outcomes for this vulnerable group in many settings. The MA-CoV study constitutes a unique opportunity to improve the understanding of the effects of COVID-19 in pregnancy in pregnant women, while it will also assess other potential mechanisms of SARS-CoV-2 transmission such as vertical transmission during pregnancy and through breastfeeding. Thus, this study has the potential to produce an immediate beneficial public health impact at both regional and global level.

Limitations

The COVID-19 pandemic scenario presents several barriers and challenges not only due to the disease itself but also due to the implementation of containment measures such as quarantine, social distancing and community containment. To avoid this situation, relevant stakeholders will be meaningfully engaged from the very beginning and all throughout the process. Moreover, local and national health authorities' recommendations are being issued on the basis of ensuring continued provision of antenatal care, HIV prevention, testing, and treatment services.

Southern Mozambique is an area with high rates of population movement between countries such as Eswatini and South Africa, where women represent a large part in some areas.[24] Thus, some participants may be lost to follow-up despite efforts to reduce bias. However, this will be considered during the data analysis. In case of high rates of participants lost to follow-up, we will conduct analyses between the baseline characteristics of retained and lost participants.

CONCLUSION

The MA-CoV project will fill knowledge gaps thanks to its prospective study design which enables assessing both prevalence and incidence of the infection and its health effects during pregnancy. The findings of this project will contribute to the understanding of the impact of SARS-CoV-2 and COVID-19 among pregnant women living in SSA countries where diseases such as malaria and HIV are highly prevalent.

ETHICS AND DISSEMINATION

The study is conducted in accordance with the European Medicines Agency (EMA)/International Council for Harmonization (ICH) Guideline on Good Clinical Practice and in total agreement with the applicable inter-national, European Union (EU) and national law of all the participating countries.46 The study protocol (V.1.0, 18 August 2021) and the informed consent forms have been reviewed and approved by the institutional and national ethics committees of Gabon (077/2021/CNE/SG/P) and Mozambique (61/CBNS/22) and the Hospital Clinic of Barcelona (HCB/2021/0942, Spain). The study is registered on clinicaltrials.gov (NCT05303168). The findings of the study will be submitted for publication in a peer-reviewed journal within 12 months of study completion through an open access mechanism, or otherwise made available publicly in compliance with H2020 open access requirements. Primary project raw data will be published in the project website.[25] At no stage will data containing personal information of research participants be released. After concluding the study's data analysis, findings will be made available to all partners, key stake-holders and Ministries of Health. The project members will actively disseminate information to the scientific community through reports, presentations at scientific forums and publications in international open-access journals.

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Authors contributions: RG conceived and designed the study; RG and AF-R wrote the study protocol. TN, GM-N, JM, ME, MR, SS, FS, and CM gave inputs to protocol methodology. AF-R, RG, GM-N, TN, MV, AM, MM, LM-N and BM were responsible for study conduct, reporting and acquisition of data. AF and RG wrote the draft manuscript, all authors reviewed the draft and read and approved the final manuscript.

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References

1. WHO Coronavirus (COVID-19) Dashboard. Available: https://covid19.who.int [Accessed 28 July 2022].
2. Knight M, Bunch K, Vousden N, et al. Characteristics and outcomes of pregnant women admitted to hospital with confirmed SARS-CoV-2 infection in UK: national population based cohort study. *BMJ* 2020;369:m2107.
3. Wastnedge EAN, Reynolds RM, van Boeckel SR, et al. Pregnancy and COVID-19. *Physiol Rev* 2021;101(1):303-18.
4. Regan AK, Arah OA, Fell DB, et al. SARS-CoV-2 Infection During Pregnancy and Associated Perinatal Health Outcomes: A National US Cohort Study. *J Infect Dis* 2022;225(5):759-67.
5. Allotey J, Stallings E, Bonet M, et al. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis. *BMJ* 2020;370:m3320.
6. Villar J, Ariff S, Gunier RB, et al. Maternal and Neonatal Morbidity and Mortality Among Pregnant Women With and Without COVID-19 Infection: The INTERCOVID Multinational Cohort Study. *JAMA Pediatr* 2021;175(8):817-26.
7. Schwartz DA, Dhaliwal A. Coronavirus Diseases in Pregnant Women, the Placenta, Fetus, and Neonate. *Adv Exp Med Biol* 2021;1318:223-41. doi: 10.1007/978-3-030-63761-3_14
8. Schwartz DA. An Analysis of 38 Pregnant Women with COVID-19, Their Newborn Infants, and Maternal-Fetal Transmission of SARS-CoV-2: Maternal Coronavirus Infections and Pregnancy Outcomes. *Arch Pathol Lab Med* 2020.
9. Ordi J, Bardaji A, Castillo P, et al. Grading schemes for placental malaria. *J Infect Dis* 2011;203(11):1694-5; author reply 95-6.
10. Ssentongo P, Heilbrunn ES, Ssentongo AE, et al. Epidemiology and outcomes of COVID-19 in HIV-infected individuals: a systematic review and meta-analysis. *Sci Rep* 2021;11(1):6283. doi: 10.1038/s41598-021-85359-3 [published Online First: 20210318]
11. Hoffmann C, Casado JL, Harter G, et al. Immune deficiency is a risk factor for severe COVID-19 in people living with HIV. *HIV Med* 2021;22(5):372-78.
12. Gupta H, Macete E, Bulo H, et al. Drug-Resistant Polymorphisms and Copy Numbers in Plasmodium falciparum, Mozambique, 2015. *Emerging infectious diseases* 2018;24(1):40-48.
13. Gonzalez R, Nhampossa T, Mombo-Ngoma G, et al. Evaluation of the safety and efficacy of dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in HIV-infected pregnant women: protocol of a multicentre, two-arm, randomised, placebo-controlled, superiority clinical trial (MAMAH project). *BMJ Open* 2021;11(11):e053197.

14. Global Technical Strategy for Malaria 2016-2030. *World Health Organization* 2015. Available: https://www.who.int/docs/default-source/documents/global-technical-strategy-for-malaria-2016-2030.pdf?sfvrsn=c82afcc_0 [accessed 20 July 2022].
15. Gonzalez R, Munguambe K, Aponte J, et al. High HIV prevalence in a southern semi-rural area of Mozambique: a community-based survey. *HIV Med* 2012;13(10):581-8.
16. Manego RZ, Mombo-Ngoma G, Witte M, et al. Demography, maternal health and the epidemiology of malaria and other major infectious diseases in the rural department Tsamba-Magotsi, Ngounie Province, in central African Gabon. *BMC Public Health* 2017;17(1):130.
17. FDA. Atellica IM SARS-CoV-2 Total (COV2T). *FDA* 2021. Available: https://www.fda.gov/media/138439/download [Accessed 1 July 2022].
18. Baud D, Greub G, Favre G, et al. Second-Trimester Miscarriage in a Pregnant Woman With SARS-CoV-2 Infection. *JAMA* 2020;323(21):2198-200.
19. Pollan M, Perez-Gomez B, Pastor-Barriuso R, et al. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. *Lancet* 2020;396(10250):535-44.
20. StataCorp 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC.
21. Nachega JB, Sam-Agudu NA, Machekano RN, et al. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection and Pregnancy in Sub-Saharan Africa: A 6-Country Retrospective Cohort Analysis. *Clin Infect Dis* 2022
22. HIV/AIDS | WHO | Regional Office for Africa. Available: https://www.afro.who.int/health-topics/hivaids [Accessed 15 May 2022].
23. Western Cape Department of Health in collaboration with the National Institute for Communicable Diseases SA. Risk Factors for Coronavirus Disease 2019 (COVID-19) Death in a Population Cohort Study from the Western Cape Province, South Africa. *Clin Infect Dis* 2021;73(7):e2005-e15.
24. IOM. World Migration Report. Geneva, 2022.
25. MA-CoV. Available: https://www.mamahproject.net/ma-cov-the-project/ [Accessed 03 June 2022].

Informed Consent of study

"Prevalence and impact of SARS-CoV-2 infection on maternal and infant health in African populations (MA-CoV)"

Introduction

The burden of COVID-19 is still unknown since access to diagnostic tests has been limited and therefore reserved for patients with severe disease and/or high-risk groups. In the African region, the number of reported cases is spreading and it is likely that vulnerable populations such as pregnant women and their foetuses will be directly and/or indirectly affected in the context of fragile health systems. It is important to understand the possible effects of COVID-19 on the health of pregnant and infants living in these regions to develop specific prevention measures.

Purpose and procedures of this study

The information coming from this MA-CoV study will help to understand the effects of the pandemic virus in African pregnant women. If you agree to be in the MA-CoV study, you will have a test done at the ***first antenatal care visit and in follow up visits*** in case you have symptoms or signs suggestive of COVID-19.

About the COVID-19 test

The test is a procedure called nasopharyngeal (NP) swab. The NP swab involves placing a swab (like a very long Q-tip) in your nose to collect cells and secretions. The swab will go into your nasal cavity, above the roof of your mouth. In some cases, the swab may only go into the nostril. The swab will be sent to a laboratory for testing to see if you are infected with COVID-19. The results of the COVID-19 testing will be made available to you, together with sufficient information to understand what the results mean. In case you are found to be infected, you will receive treatment free of charge and information regarding isolation and transmission prevention measures to be put in place at your home.

What happens during the study?

If you agree to be in this study, your first visit will continue today, after you read, discuss, and sign or put thumbprint on this form. You will be asked to come back to the clinic monthly before delivery. In addition, you must agree to deliver your baby at the study facility rather than at home.

If you agree to be in this study:

- We will first ask you some questions about yourself and your health
- We will ask you to give information on where you live and how to keep in contact with you
- A study clinician will examine you and will check your pregnancy status
- You will also be asked to give a venous blood sample at the first visit for tests of your blood (malaria and COVID-19 virus antibodies)
- In case you will be unwell with malaria or other infection, you will have additional blood tests done and if needed you will be given medicine and asked to come back here as scheduled by study staff
- You and your baby will receive a unique identification number (ID) and identification study card, which you will be requested to present to the study staff at every visit
- At delivery you will be visited during in the labour ward and you and your new-born baby will be examined by the study personnel.
- In addition to venous blood being collected from you, also a sample of cord blood will be taken to analyse the presence of COVID-19 virus antibodies
- A piece of placenta will be examined at the study laboratory and also tested for COVID-19 virus
- You must agree to deliver at the health facility but in case you deliver at home, the study staff will visit you as soon as possible but not later than one week after delivery and will ask you questions about your delivery and about health of your infant. At this visit you and your infant will be examined by the study personnel. Blood sample will be taken from you for tests of malaria

- We will ask you to provide us with a small sample of breastmilk (3 ml, less than a teaspoon) within three days and one month after your infant's birth to investigate if the virus can be found in maternal milk.
- When your baby is born, your child will be followed up until he/she is 1 month old
- You will be asked to come back with your new-born to the study clinic around 1 month after delivery to exam your baby and see if your baby is growing well

Other COVID-19 analyses and samples

We will also analyse the presence of the virus (which is called SARS-CoV-2) in the blood and placental samples that will be collected from you at enrolment and at the end of pregnancy. In case you are found to be infected with the COVID-19 virus, your infant will also be tested with a NP swab at birth. Also, if she/he presents with symptoms or signs suggestive of COVID-19 during her/his first month of life, she/he will have a test done and will receive the indicated treatment.

Alternatives to joining the MA-CoV study

If you choose not to participate in this study you will receive standard ANC care as before.

Risks or discomforts *(mother and infant)*

You might feel slight discomfort when we take nasopharyngeal swabs or venous blood samples at enrolment and delivery. There will be no other risks.

Benefits to you and your infant

By participating in the study, you may get better diagnosis of COVID-19 and other diseases such as malaria because of increased number of tests done. You and your baby will be regularly seen by clinical staff and in case of any symptoms or abnormal test results you and your baby will be either treated here or referred to another clinic for medical care.

STATEMENT of CONSENT AND SIGNATURE

Participant approval:
The consent form has been explained to me and I agree to take part in the MA-CoV study. I understand that I am free to choose to be in this activity and that saying "No" will not affect the treatment I get in this clinic, now and in future.
NOTE: You are not giving up any of your legal rights by signing this informed consent document.

If you agree circle YES

Volunteer's Name (print)	Volunteer's Signature or Thumbprint (if cannot write)	Date
Volunteer's Legal Guardian or Representative (as per country policy) (print)	Legal Guardian's Signature	Date
Witness's Name (if participant illiterate) (print)	Witness's Signature	Date

I have explained the purpose of this study to the volunteer. To the best of my knowledge, she understands the purpose, procedures, risks and benefits of this study.

Investigator/Designee Name (print)	Investigator/Designee Signature	Date

NOTE: This consent form with original signatures must be retained on file by the principal investigator. A copy must be given to the volunteer. If the woman refuses to take her copy of the consent with her, she states so below and signs and dates her decline statement.

BMJ Open

Prevalence and impact of SARS-CoV-2 infection on maternal and infant health in African populations: protocol of a multi-centre prospective cohort study (MA-CoV project)

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Prevalence and impact of SARS-CoV-2 infection on maternal and infant health in African populations: protocol of a multi-centre prospective cohort study (MA-CoV project)

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ABSTRACT

Introduction. Pregnant women are currently considered a vulnerable population to SARS-CoV-2 infection, with increased risk of severe COVID-19, pre-term birth and maternal mortality. There is, however, a paucity of data on the burden of maternal SARS-CoV-2 infection in sub-Saharan countries. The objective of this study is to determine the prevalence and health effects of maternal SARS-CoV-2 infection in selected sites from Gabon and Mozambique.

Methods and analysis. MA-CoV (MAternal CoVid) is an observational, multicenter, prospective cohort study where 1000 pregnant women (500 per country) will be enrolled at the antenatal clinic visits. Participants will undergo monthly follow-up at each antenatal care visit, delivery and post-partum visit. The primary study outcome is the prevalence of SARS-CoV-2 infection during pregnancy. The clinical presentation of COVID-19 in pregnancy will also be characterized, and incidence of infection during pregnancy will be evaluated, as well as the risk factors of maternal and neonatal morbidity and mortality associated with SARS-CoV-2 infection and the risk of mother-to-child transmission of SARS-CoV-2. SARS-CoV-2 infection screening will be performed through polymerase chain reaction (PCR) diagnosis.

Ethics and dissemination. The protocol was reviewed and approved by the *Comité National d'Éthique pour la Recherche au Gabon*, *Comité Nacional de Bioética para Saúde de Moçambique* and the Ethics Committee of the Hospital Clinic of Barcelona (Spain). Project results will be presented to all stakeholders and published in open-access journals.

Registration number. NCT05303168.

Strengths and limitations

- The prospective longitudinal study design which covers the pregnancy and post-partum periods will allow us to assess both prevalence and incidence of SARS-CoV-2 infection and its health effects in pregnancy and perinatal outcomes.
- The inclusion of participants from two different sub-Saharan countries will provide information on the differences in the distribution of SARS-CoV-2 infection.
- The COVID-19 pandemic scenario presents several barriers and challenges not only due to the disease itself but also due to the implementation of containment measures such as quarantine, social distancing and community containment. This may affect antenatal care attendance, as well as hinder the implementation of the study.
- As COVID-19 vaccination of study participants may limit our capacity to estimate the prevalence of the infection through SARS-CoV-2 serology, we will assess the prevalence of antibodies against the SARS-CoV-2 nucleocapsid protein, which reflects past SARS-CoV-2 infection.

Keywords: SARS-CoV-2, sub-Saharan Africa, pregnancy

INTRODUCTION

As of July 2022, more than nine million COVID-19 cases and 170,000 related deaths have been reported in the World Health Organization (WHO) African region, while only 21% of the African population has been fully vaccinated.[1] However, the real burden of SARS-CoV-2 in Africa is probably still unknown and underestimated.

Pregnant women are at increased susceptibility of SARS-CoV-2 infection, particularly those with co-morbidities such as preeclampsia and gestational diabetes mellitus.[2] This may be explained by the pregnancy-induced changes, which include a decreased lung volume, an increased risk for thromboembolic disease and immunological changes in order to allow for the growth of a semi-allogenic fetus.[3] Effects of SARS-CoV-2 infection on maternal and neonatal health include increased risk of admission to intensive care and need of mechanical ventilation, induced abortion, c-section, pre-term birth, foetal growth restriction, post-partum hemorrhage and maternal mortality. [4-6]

Besides, mother to child transmission of SARS-CoV-2 is possible intrauterine, intrapartum, and at the postpartum period.[7] Several studies have reported the detection of SARS-CoV-2 in the fetal side of the placenta, indicating transplacental foetal infection.[7] Of note, most of the evidence of these effect has been gathered in high income countries.

In Sub-Saharan Africa (SSA), SARS-CoV-2 overlaps geographically with endemic infectious diseases such as the Human Immunodeficiency Virus (HIV) and malaria in a context of low SARS-CoV-2 vaccination coverage. For instance, co-infection with SARS-CoV-2 and malaria in pregnant women, might have deleterious effects in the foetal development, considering the reported inflammatory and histologic changes at the placental level found in both infections.[8 9] Additionally, there is evidence that immunosuppressed HIV-infected individuals are at increased risk of severe COVID-19 and death than non-infected individuals.[10 11] Importantly, the burden of HIV infection is concentrated in SSA.[12]

The information on the burden of SARS-CoV-2 infection in pregnancy in SSA countries is very limited. As to date, most of studies have been carried out in high income countries, neglecting the particular characteristics of SARS-CoV-2 infection in pregnancy in low-middle income countries. In this context, the present study was developed leveraging on an ongoing multicenter, two-arm, placebo-controlled, individually randomized trial aiming to assess the efficacy and safety of dihydroartemisinin-piperaquine as intermittent preventive treatment for HIV-infected pregnant women (NCT03671109).[13]

Study aims and hypotheses

The primary objective of the MA-CoV (Maternal CoVid) study is to determine the prevalence and incidence of SARS-CoV-2 infection during pregnancy. Secondary objectives include to describe the effects of maternal SARS-CoV-2 infection on pregnancy and perinatal outcomes, to characterize the clinical features of COVID-19 disease in pregnancy, and to assess the potential vertical transmission and through breastfeeding of SARS-CoV-2 from infected mothers to their offspring. The main study hypotheses are: (1) SARS-CoV-2 infection during pregnancy may influence maternal and perinatal outcomes, (2) SARS-CoV-2 clinical manifestations may be different in pregnant women compared to non-pregnant adults, and (3) SARS-CoV-2 can be transmitted from mother to child prenatally and postnatally.

METHODS AND ANALYSIS

MA-CoV is an observational, multicenter prospective cohort study.

Study settings

The study will be carried out in Libreville and Lambaréné (Gabon), and in Manhiça (Mozambique). SARS-CoV-2 reported cases ranged from 48,000 in Gabon to 228,000 in Mozambique as per July 2022.[14] Additionally, HIV prevalence among pregnant women ranges from 6% in study sites of Gabon to 29% in study sites of Mozambique.[15] [16] Malaria epidemiological indicators and SARS-CoV-2 and HIV prevalence in pregnancy in study sites are shown in table 1.

Table 1. SARS-CoV-2, malaria and HIV epidemiology in study countries

Country	Site	SARS-CoV-2 reported cases (country-level)	*P.falciparum* infection prevalence in women at delivery†	HIV prevalence in pregnant women
Mozambique	Manhiça	228,000[1]	6%	29%[15]
Gabon	Lambaréné	48,000[1]	11%	6%[16]
	Libreville		NI	6%[16]

†Data from 2010-2012 in women receiving either two IPTp doses of mefloquine or SP (Tuikue-Ndam et al, unpublished). NI: No information; MTCT: mother-to-child transmission

Study population

All pregnant women attending the study antenatal care (ANC) services will be screened for participation in the study. Inclusion criteria are: (1) permanent resident in the study area and (2) willing to deliver in the study maternity wards. Pregnant women planning to move out the study area in the following 7 months from enrolment will be excluded.

Informed consent and recruitment

All participants will receive information about study procedures. A signed informed consent form (or thumb-printed with a witness if the woman is illiterate) will be obtained before any study procedures are carried out by study nurses in each site. The informed consent will cover the woman and the new born infant. The study's informed consent is available as Supplementary Material file 1. If the participant is under the legal age of maturity, she will sign the assent form and her legal guardian will sign the informed consent according to national ethics local policies.

After the study details are explained and informed consent is signed, a study identification card containing the individual study number and basic demographic information will be given to the participant in order to facilitate identification at all study contacts.

Follow-up and measurement of outcomes

At baseline, the woman's demographic and obstetric information will be recorded in study specific case report forms (CRFs) (Supplementary material file 2)

Physical and clinical examination at enrolment

The physical examination of the woman will include the following assessments: weight, height, gestational age by bimanual palpation and measurement of middle-upper arm circumference (MUAC). Ultrasound will be performed to determine gestational age and confirm pregnancy viability at enrolment if possible. COVID-19 suggestive symptoms will be assessed, and should

the woman present them, A nasopharyngeal swab will be collected for detection of SARS-CoV-2 viral RNA. Additionally, a nasopharyngeal swab will be collected in a sub-sample of 100 study participants regardless presence of COVID-19 symptoms for screening of SARS-CoV-2 infection.

Baseline biological samples

At enrolment, a venous blood sample (5 mL) will be collected for analysis of hemoglobin level, SARS-CoV-2 total antibodies, malaria polymerase chain reaction (PCR) (if the woman presents malaria-suggestive symptoms), and HIV viral load and CD4 cell count (if the woman is HIV-infected).

Antenatal follow-up

Participants will receive the standard ANC package of interventions, which includes intermittent preventive treatment (IPTp) of malaria, iron and folate supplementation, following national guidelines. During monthly ANC visits. COVID-19 suggestive symptoms will be assessed, and should the woman present them, a PCR to detect SARS-CoV-2 viral load will be performed.

Unscheduled visits

Study participants reporting being sick at the health facilities (including suspicion of COVID-19) will be seen by study personnel. Every unscheduled visit of the woman from enrolment until the post-partum visit will be recorded into a study CRF.

End of pregnancy and post-partum period

At the end of pregnancy, 5 ml of maternal blood sample will be collected for analysis of antibodies (IgG and IgM) against SARS-CoV-2, malaria parasitaemia and HIV viral load (in case the woman is HIV-infected). Additionally, whenever possible, cord blood and placental tissue samples will be collected for SARS-CoV-2 serologic and PCR analysis, respectively.

Breastmilk samples (3 ml) will also be collected within the first three days after delivery (colostrum) and at the post-partum visit (approximately six weeks after the end of pregnancy), for detection of SARS-CoV-2 by PCR. In addition, a neonatal throat swab will be collected at birth for SARS-CoV-2 analysis by PCR in infants born to COVID-19 positive mothers. A summary of study procedures is displayed in Table 2.

Table 2. Study visits and procedures schedule

Study procedure	First ANC clinic visit	Routine ANC clinic visits	End of pregnancy	1 month after end of pregnancy	Unscheduled visits	Infant Assessment (birth and 1 month)
Inclusion/ Exclusion criteria check	X					
Written informed consent	X					
Demographics, socio-economic/ Medical history	X				X	
COVID-19 screening#	X	X			X	X
Record of medications/ Morbidity	X	X	X	X	X	
Physical examination/clinical	X		X		X	
Gestational age	X	X	X		X	
Temperature				X	X	X
Blood Pressure	X		X	X	X	
Weight	X	X		X	X	
Height	X					
MUAC	X			X		
Presence of proteins in urine	X					
CD4 count*	X					
HIV viral load*	X		X			
SARS-CoV-2 serology	X		X			
Malaria blood PCR	X					
Blood smear	†	†	X	X	†	
Haemoglobin test	X		X	X		
Peripheral venous blood (mother)	X		X			
Cord blood			X			
Placental biopsy			X			
Placental impression smears			X			
Breastmilk (SARS-CoV2)			X	X		

ANC: Antenatal care; MUAC: middle-upper circumference

In participants with suggestive symptoms/signs of COVID-19 (fever, cough, shortness of breath, sudden onset of anosmia, ageusia or dysgeusia), except in a sub-sample of 100 participants at enrolment among whom it will be performed regardless of presence of symptoms.

*Only in HIV-infected women

† Only in women passively reporting sick AND presenting with malaria related signs/symptoms (fever (≥37,5º C) or having history of fever in the past 24 hours, arthromyalgia or headache), as per national management guidelines

Infant assessment

A neonatal throat swab will be collected at birth for SARS-CoV-2 analysis by PCR in infants born to COVID-19 positive mothers. Should the neonate present with symptoms and/or signs suggestive of acute respiratory infection during the first month of life, another throat swab will be collected for SARS-CoV-2 testing by PCR.

Laboratory tests

Detection of SARS-CoV-2

A real-time polymerase chain reaction (RT-PCR) COVID-19 assay diagnostic test will be performed at the study laboratories for detection of SARS-CoV-2 viral RNA. Real-Time PCR technology utilizes polymerase chain reaction for the amplification of specific target sequences and target specific probes for the detection of the amplified RNA. The probes are labelled with fluorescent reporter and quencher dyes.

The Elecsys® Anti-SARS-CoV-2 and Elecsys® Anti-SARS-CoV-2 S essays (Roche Diagnostics) will be used for detection of total anti-SARS-CoV-2 spike (S) and nucleocapsid (N) antibodies through electro-chemiluminescent immunoassays (ECLIA) intended for the qualitative detection of total antibodies (including IgG and IgM) to SARS-CoV-2 in human serum and plasma.[17 18] This assay is a double-antigen sandwich electrochemiluminescence immunoassay, which separates bound from unbound substances with streptavidin-coated microparticles before applying a voltage to the electrode.[17 18] ***Malaria parasitological and haematological determinations***

In case of malaria suspicion, thick and thin blood smears will be collected and stained with Giemsa's stain and examined for *Plasmodium spp*. following standard procedures. Also, blood haemoglobin will be determined following local SOPs.

Detection of HIV and quantitative determination of viral load

In HIV-infected women, quantitative PCR HIV viral load will be determined from the venous blood samples drawn at enrolment and at delivery. HIV viral load will be determined from plasma cryopreserved at -80ºC using the devices in place *in the study sites (such as* COBAS® AMPLICOR, AmpliPrep [Roche Diagnostics] *or GeneXpert).*

Immunological determinations related to HIV status

In HIV-infected women, CD4+T cell count will be determined by flow cytometry after staining of whole blood with CD3, CD8 and CD4 fluorochrometolabelled antibodies and acquisition using FACSCalibur (BD Biosciences) and TruCOUNT tubes (Becton Dickinson, San Jose, CA; USA) *or MiniVIdas device*.

Placental samples analysis

A placental sample will be collected for malaria histological analysis. The biopsies will be immediately placed in 25 mL of 10% neutral buffered formalin and kept at 4ºC until processed and embedded in paraffin wax by standard techniques. Paraffin sections will be stained with haematoxylin and eosin, Giemsa's stain and the periodic acid-Schiff technique. Placental histology will include the examination of inflammatory signs (such as presence of neutrophils and monocytes) in the subchorial space and the umbilical cord connective tissue (funisitis) and analysis of intervillous fibrin deposition.[19]

Additionally, another placental sample will be collected for SARS-CoV-2 histopathological detection. The placental tissue will be placed in a sterile 150 ml bottle and kept in a -80ºC freezer until the sample is processed. Placental histology will include the examination of inflammatory signs (such as presence of neutrophils and monocytes) in the subchorial space and the umbilical cord connective tissue (funisitis) and analysis of intervillous fibrin deposition.[19]

Data management

All the data will be collected using paper CRFs during the study visits, from interviews and clinical observation or measures taken to participants. Results from the laboratory analyses performed in collected participant's biological samples will also be collected and entered in the CRFs.

Data from the study source document will be double entered into the study database using the OpenClinica open source software version 3.1.4 (Copyright OpenClinica LLC and collaborators, Waltham, MA, USA, www.OpenClinica.com) Subsequently, entered data will be systematically checked by Data Management team using error messages printed from validation programs and database listings. Quality control audits of all key safety and efficacy information in the database will be made prior to locking the database.

Study outcomes

The primary outcome of the study will be the prevalence of anti-SARS-CoV-2 N protein total antibodies at delivery. The secondary endpoints can be found in Table 3.

Table 3. Study endpoints

Primary endpoint	Prevalence of anti-SARS-CoV-2 N protein antibodies (Ig G and/or Ig M positive) against SARS-CoV-2 among pregnant women at delivery
Secondary endpoints	1. PCR-confirmed SARS-CoV-2 infection among pregnant women at recruitment 2. Incidence of SARS-CoV-2 infection during pregnancy 3. Maternal and neonatal morbidity and mortality due to SARS-CoV-2 infection during pregnancy 4. Pregnancy and perinatal adverse outcomes 5. Rate of vertical transmission of SARS-CoV-2 from infected mothers to their offspring, during the prenatal and perinatal period 6. CD4 cell counts and HIV viral load 7. Malaria parasitaemia at delivery (from maternal sample collected at delivery)

Sample size

Considering six months of enrolment and recruitment rates of participants in ongoing clinical trials in the two study sites, it was expected to include approximately 1000 women in the study. Assuming a 5% prevalence of SARS-CoV-2 of infection during pregnancy, this sample size would allow estimating the proportion of women with the infection with a 1.4% precision at the 95% confidence level.[20]

Statistical analysis

Infection by SARS-CoV-2 will be defined by presence of anti-SARS-CoV-2 nucleocapsid (N) protein antibodies (total IgG, IgG and/or IgM) or by a positive SARS-CoV-2 PCR. Asymptomatic COVID-19 infection will be defined by presence of SARS-CoV-2 nucleocapsid (N) antibodies and/or a positive COVID-19 PCR without COVID-19 associated symptoms. Women with baseline anti-SARS-CoV-2 N protein SARS-CoV-2 antibodies will be considered infected before study enrolment.

The socio-demographic characteristics of the study participants will be described using summary statistics. Continuous variables will be summarized using mean or median (depending on the distribution of the variable) and standard deviation or interquartile range. Categorical variables will be described using frequencies and percentages. Proportions for categorical variables will be assessed using the chi-square test or Fisher's exact test where appropriate. The Student's t test or Wilcoxon rank-sum test will be used to compare means and medians, respectively, of

continuous variables according to variable characteristics. Only records with information on the outcome of interest will be analyzed.

Incidences of all-cause hospital admissions and all-cause outpatient attendance during pregnancy will be analyzed using negative binomial regression and compared by SARS-CoV-2 infection status. The incidence of COVID-19 and clinical malaria episodes will be determined. The frequency of COVID-19 will be compared between HIV-infected and HIV-uninfected women using a negative binomial regression. The proportion of women with adverse pregnancy outcomes will be compared by SARS-CoV-2 infection status using a modified binomial regression. These analyses will be done unadjusted and adjusted by baseline significant variables (age, gestational age, gravidity, RPR, anaemia and literacy, study intervention) and clinically relevant factors depending on the outcome for control of confounding factors. Incidences of hospital admissions in the neonate will be also analyzed using negative binomial regression. Data analysis will be performed using Stata (Stata Corp).[21]

Patient and public involvement. Patients will not be directly involved in the design, conduct, reporting or dissemination plans of the study.

DISCUSSION

At the onset of the COVID-19 pandemic, the extent of the risks in pregnancy was uncertain. In this context, the MA-CoV study was conceived to address fundamental questions on the burden and effects of SARS-CoV-2 infection during pregnancy. MA-CoV is an international, prospective observational cohort study that plans to follow pregnant women living in study areas of Gabon and Mozambique, where malaria and HIV infections are endemic and the real burden of SARS-CoV-2 infection is still unknown. Participants will be followed at monthly ANC visits, until 6 weeks after end of pregnancy. Additionally, the presence of antibodies (IgG/IgM) against SARS-CoV-2 in blood samples will be determined. The clinical presentation of COVID-19 in pregnancy will also be characterized, and incidence of infection during pregnancy will be evaluated, as well as the risk factors of maternal and neonatal morbidity and mortality associated with SARS-CoV-2 infection and the risk of mother-to-child transmission of SARS-CoV-2. Recruitment is expected to finish in September 2022, while patient follow-up is expected to be completed in April 2023.

The effects of SARS-CoV-2 infection on maternal and neonatal health have been described mostly by studies performed in high income countries, and include increased risk of admission to intensive care, abortion, c-section, pre-term birth, foetal growth restriction, post-partum hemorrhage and maternal mortality. [4-6] A retrospective cohort study analyzing routine data that was performed in six SSA countries reported similar findings.[22] In addition, interaction of COVID-19 with other global epidemics such as HIV is particular relevant in the African region, given that it has the highest world incidence of HIV infection, being women of reproductive age at higher risk.[23] Recent studies have shown that HIV infection is associated with a significant increased risk of contracting SARS-CoV-2. In addition, immunosuppressed HIV-infected individuals have been shown to have higher incidence of severe COVID-19 and death than non-HIV-infected individuals. [10 11 24] Importantly, the study performed in six countries of SSA found that pregnant women with HIV had an increased risk of admission to intensive care.[22]

Pregnant women still face disproportionate inequalities in access to and quality health care. The most essential maternal and reproductive health interventions do not reach yet the poorest and most vulnerable women, girls and children in the developing world. This results in marked poor understanding of the particular characteristics and health outcomes for this vulnerable group in many settings. The MA-CoV study constitutes a unique opportunity to improve the

understanding of the effects of COVID-19 in pregnancy in pregnant women, while it will also assess other potential mechanisms of SARS-CoV-2 transmission such as vertical transmission during pregnancy and through breastfeeding. Thus, this study has the potential to produce an immediate beneficial public health impact at both regional and global level.

Limitations

The COVID-19 pandemic scenario presents several barriers and challenges not only due to the disease itself but also due to the implementation of containment measures such as quarantine, social distancing and community containment. To avoid this situation, relevant stakeholders will be meaningfully engaged from the very beginning and all throughout the process. Moreover, local and national health authorities' recommendations are being issued on the basis of ensuring continued provision of antenatal care, HIV prevention, testing, and treatment services.

Southern Mozambique is an area with high rates of population movement between countries such as Eswatini and South Africa, where women represent a large part in some areas.[25] Thus, some participants may be lost to follow-up despite efforts to reduce bias. However, this will be considered during the data analysis. In case of high rates of participants lost to follow-up, we will conduct analyses between the baseline characteristics of retained and lost participants.

ETHICS AND DISSEMINATION

The study is conducted in accordance with the European Medicines Agency (EMA)/International Council for Harmonization (ICH) Guideline on Good Clinical Practice and in total agreement with the applicable inter-national, European Union (EU) and national law of all the participating countries.46 The study protocol (V.1.0, 18 August 2021) and the informed consent forms have been reviewed and approved by the institutional and national ethics committees of Gabon (077/2021/CNE/SG/P) and Mozambique (61/CBNS/22) and the Hospital Clinic of Barcelona (HCB/2021/0942, Spain). The study is registered on clinicaltrials.gov (NCT05303168). The findings of the study will be submitted for publication in a peer-reviewed journal within 12 months of study completion through an open access mechanism, or otherwise made available publicly in compliance with H2020 open access requirements. Primary project raw data will be published in the project website.[26] At no stage will data containing personal information of research participants be released. After concluding the study's data analysis, findings will be made available to all partners, key stake-holders and Ministries of Health. The project members will actively disseminate information to the scientific community through reports, presentations at scientific forums and publications in international open-access journals.

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Authors contributions: RG conceived and designed the study; RG and AF-R wrote the study protocol. TN, GM-N, JM, ME, MR, SS, FS, and CM gave inputs to protocol methodology. AF-R, RG, GM-N, TN, MV, AM, MM, LM-N and BM were responsible for study conduct, reporting and acquisition of data. AF-R and RG wrote the draft manuscript, all authors reviewed the draft and read and approved the final manuscript.

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Competing interests: None declared

References

1. WHO Coronavirus (COVID-19) Dashboard. Available: https://covid19.who.int [Accessed 28 July 2022].
2. Knight M, Bunch K, Vousden N, et al. Characteristics and outcomes of pregnant women admitted to hospital with confirmed SARS-CoV-2 infection in UK: national population based cohort study. *BMJ* 2020;369:m2107.
3. Wastnedge EAN, Reynolds RM, van Boeckel SR, et al. Pregnancy and COVID-19. *Physiol Rev* 2021;101(1):303-18.
4. Regan AK, Arah OA, Fell DB, et al. SARS-CoV-2 Infection During Pregnancy and Associated Perinatal Health Outcomes: A National US Cohort Study. *J Infect Dis* 2022;225(5):759-67.
5. Allotey J, Stallings E, Bonet M, et al. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis. *BMJ* 2020;370:m3320.
6. Villar J, Ariff S, Gunier RB, et al. Maternal and Neonatal Morbidity and Mortality Among Pregnant Women With and Without COVID-19 Infection: The INTERCOVID Multinational Cohort Study. *JAMA Pediatr* 2021;175(8):817-26.
7. Schwartz DA, Dhaliwal A. Coronavirus Diseases in Pregnant Women, the Placenta, Fetus, and Neonate. *Adv Exp Med Biol* 2021;1318:223-41.
8. Schwartz DA. An Analysis of 38 Pregnant Women with COVID-19, Their Newborn Infants, and Maternal-Fetal Transmission of SARS-CoV-2: Maternal Coronavirus Infections and Pregnancy Outcomes. *Arch Pathol Lab Med* 2020.
9. Ordi J, Bardaji A, Castillo P, et al. Grading schemes for placental malaria. *J Infect Dis* 2011;203(11):1694-5; author reply 95-6.
10. Ssentongo P, Heilbrunn ES, Ssentongo AE, et al. Epidemiology and outcomes of COVID-19 in HIV-infected individuals: a systematic review and meta-analysis. *Sci Rep* 2021;11(1):6283.
11. Hoffmann C, Casado JL, Harter G, et al. Immune deficiency is a risk factor for severe COVID-19 in people living with HIV. *HIV Med* 2021;22(5):372-78.
12. Gupta H, Macete E, Bulo H, et al. Drug-Resistant Polymorphisms and Copy Numbers in Plasmodium falciparum, Mozambique, 2015. *Emerging infectious diseases* 2018;24(1):40-48.
13. Gonzalez R, Nhampossa T, Mombo-Ngoma G, et al. Evaluation of the safety and efficacy of dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in HIV-infected pregnant women: protocol of a multicentre, two-arm, randomised, placebo-controlled, superiority clinical trial (MAMAH project). *BMJ Open* 2021;11(11):e053197.
14. Global Technical Strategy for Malaria 2016-2030. *World Health Organization* 2015. Available: https://www.who.int/docs/default-source/documents/global-technical-strategy-for-malaria-2016-2030.pdf?sfvrsn=c82afcc_0 [Accessed 20 July 2022].
15. Gonzalez R, Munguambe K, Aponte J, et al. High HIV prevalence in a southern semi-rural area of Mozambique: a community-based survey. *HIV Med* 2012;13(10):581-8. doi: 10.1111/j.1468-1293.2012.01018.x [published Online First: 20120415]
16. Manego RZ, Mombo-Ngoma G, Witte M, et al. Demography, maternal health and the epidemiology of malaria and other major infectious diseases in the rural department Tsamba-Magotsi, Ngounie Province, in central African Gabon. *BMC Public Health* 2017;17(1):130. doi: 10.1186/s12889-017-4045-x [published Online First: 20170128]

17. Elecsys® Anti-SARS-CoV-2. Roche. Available: https://diagnostics.roche.com/gb/en/products/params/elecsys-anti-sars-cov-2.html [Accessed 28 February 2023].
18. Elecsys® Anti-SARS-CoV-2 S. Roche. Available: https://diagnostics.roche.com/global/en/products/params/elecsys-anti-sars-cov-2-s.html [Accessed 28 February 2023].
19. Baud D, Greub G, Favre G, et al. Second-Trimester Miscarriage in a Pregnant Woman With SARS-CoV-2 Infection. *JAMA* 2020;323(21):2198-200. doi: 10.1001/jama.2020.7233
20. Pollan M, Perez-Gomez B, Pastor-Barriuso R, et al. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. *Lancet* 2020;396(10250):535-44.
21. StataCorp. Stata Statistical Software: Release 17. : College Station, TX: StataCorp LLC., 2021.
22. Nachega JB, Sam-Agudu NA, Machekano RN, et al. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection and Pregnancy in Sub-Saharan Africa: A 6-Country Retrospective Cohort Analysis. *Clin Infect Dis* 2022
23. HIV/AIDS | WHO | Regional Office for Africa. Available: https://www.afro.who.int/health-topics/hivaids [Accessed 15 May 2022].
24. Western Cape Department of Health in collaboration with the National Institute for Communicable Diseases SA. Risk Factors for Coronavirus Disease 2019 (COVID-19) Death in a Population Cohort Study from the Western Cape Province, South Africa. *Clin Infect Dis* 2021;73(7):e2005-e15.
25. IOM. World Migration Report. Geneva, 2022.
26. MA-CoV. Available: https://www.mamahproject.net/ma-cov-the-project/ [Accessed 03 June 2022].

Prevalence and impact of SARS-CoV-2 infection on maternal and infant health in African populations: protocol of a multi-centre prospective cohort study (MA-CoV project)

Supplementary material

File 1: Informed Consent of study

"Prevalence and impact of SARS-CoV-2 infection on maternal and infant health in African populations (MA-CoV)"

Introduction

The burden of COVID-19 is still unknown since access to diagnostic tests has been limited and therefore reserved for patients with severe disease and/or high-risk groups. In the African region, the number of reported cases is spreading and it is likely that vulnerable populations such as pregnant women and their foetuses will be directly and/or indirectly affected in the context of fragile health systems. It is important to understand the possible effects of COVID-19 on the health of pregnant and infants living in these regions to develop specific prevention measures.

Purpose and procedures of this study

The information coming from this MA-CoV study will help to understand the effects of the pandemic virus in African pregnant women. If you agree to be in the MA-CoV study, you will have a test done at the ***first antenatal care visit and in follow up visits*** in case you have symptoms or signs suggestive of COVID-19.

About the COVID-19 test

The test is a procedure called nasopharyngeal (NP) swab. The NP swab involves placing a swab (like a very long Q-tip) in your nose to collect cells and secretions. The swab will go into your nasal cavity, above the roof of your mouth. In some cases, the swab may only go into the nostril. The swab will be sent to a laboratory for testing to see if you are infected with COVID-19. The results of the COVID-19 testing will be made available to you, together with sufficient information to understand what the results mean. In case you are found to be infected, you will receive treatment free of charge and information regarding isolation and transmission prevention measures to be put in place at your home.

What happens during the study?

If you agree to be in this study, your first visit will continue today, after you read, discuss, and sign or put thumbprint on this form. You will be asked to come back to the clinic monthly before delivery. In addition, you must agree to deliver your baby at the study facility rather than at home.

If you agree to be in this study:

- We will first ask you some questions about yourself and your health
- We will ask you to give information on where you live and how to keep in contact with you
- A study clinician will examine you and will check your pregnancy status
- You will also be asked to give a venous blood sample at the first visit for tests of your blood (malaria and COVID-19 virus antibodies)
- In case you will be unwell with malaria or other infection, you will have additional blood tests done and if needed you will be given medicine and asked to come back here as scheduled by study staff
- You and your baby will receive a unique identification number (ID) and identification study card, which you will be requested to present to the study staff at every visit
- At delivery you will be visited during in the labour ward and you and your new-born baby will be examined by the study personnel.
- In addition to venous blood being collected from you, also a sample of cord blood will be taken to analyse the presence of COVID-19 virus antibodies
- A piece of placenta will be examined at the study laboratory and also tested for COVID-19 virus
- You must agree to deliver at the health facility but in case you deliver at home, the study staff will visit you as soon as possible but not later than one week after delivery and will ask you questions about your delivery and about health of your infant. At this visit you and your infant will be examined by the study personnel. Blood sample will be taken from you for tests of malaria
- We will ask you to provide us with a small sample of breastmilk (3 ml, less than a teaspoon) within three days and one month after your infant's birth to investigate if the virus can be found in maternal milk.
- When your baby is born, your child will be followed up until he/she is 1 month old
- You will be asked to come back with your new-born to the study clinic around 1 month after delivery to exam your baby and see if your baby is growing well

Other COVID-19 analyses and samples

We will also analyse the presence of the virus (which is called SARS-CoV-2) in the blood and placental samples that will be collected from you at enrolment and at the end of pregnancy. In case you are found to be infected with the COVID-19 virus, your infant will also be tested with a NP swab at birth. Also, if she/he presents with symptoms or signs suggestive of COVID-19 during her/his first month of life, she/he will have a test done and will receive the indicated treatment.

Alternatives to joining the MA-CoV study

If you choose not to participate in this study you will receive standard ANC care as before.

Risks or discomforts *(mother and infant)*

You might feel slight discomfort when we take nasopharyngeal swabs or venous blood samples at enrolment and delivery. There will be no other risks.

Benefits to you and your infant

By participating in the study, you may get better diagnosis of COVID-19 and other diseases such as malaria because of increased number of tests done. You and your baby will be regularly seen by clinical staff and in case of any symptoms or abnormal test results you and your baby will be either treated here or referred to another clinic for medical care.

STATEMENT of CONSENT AND SIGNATURE

Participant approval:
The consent form has been explained to me and I agree to take part in the MA-CoV study. I understand that I am free to choose to be in this activity and that saying "No" will not affect the treatment I get in this clinic, now and in future.
NOTE: You are not giving up any of your legal rights by signing this informed consent document.

If you agree circle YES

Volunteer's Name (print)	Volunteer's Signature or Thumbprint (if cannot write)	Date

Volunteer's Legal Guardian or Representative (as per country policy) (print)	Legal Guardian's Signature	Date

Witness's Name (if participant illiterate) (print)	Witness's Signature	Date

I have explained the purpose of this study to the volunteer. To the best of my knowledge, she understands the purpose, procedures, risks and benefits of this study.

Investigator/Designee Name (print)	Investigator/Designee Signature	Date

NOTE: This consent form with original signatures must be retained on file by the principal investigator. A copy must be given to the volunteer. If the woman refuses to take her copy of the consent with her, she states so below and signs and dates her decline statement.

File 2: Study case report forms

ISGlobal Barcelona Institute for Global Health MA-CoV Maternal health and COVID-19	**ID** MACOC- |__|-|__|__|__|__| Site Code Subject Number **Participant's initials** |__|__| |__| 1. 2. Family name

Prevalence and impact of SARS-CoV-2 infection on maternal and infant health in African populations (MA-CoV)

Project Acronym	MA-CoV

eCRF book ID MA-CoV
Version: v.3.1
Date: 28th April 2022

BIRTH

Newborn visit

Ma-COV **Event: Birth - Newborn visit**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

	INCLUSION CRITERIA CHECK	
1	Mother's ID	MACOW|__|-|__|__|__|__| Site Code Subject Nº
2	Date of birth	|__|__| - |__|__|__| - |__|__|__|__| Day Month Year
3	Sex	Masculine ☐ Feminine ☐
	MEDICAL HISTORY AND PHYSICAL EXAMINATION AT BIRTH	
4	Weight (g)	|__|__|__|__|__|
5	Length (cm)	|__|__|.|__|
6	Head circumference (cm)	|__|__|.|__|
7	Axillary temperature (ºC)	|__|__|.|__|
8	Congenital abnormalities?	Yes ☐ No ☐
	8.1. Face and head	Normal ☐ Abnormal ☐ Unknown ☐
	8.2. Limbs	Normal ☐ Abnormal ☐ Unknown ☐
	8.3. Chest	Normal ☐ Abnormal ☐ Unknown ☐
	8.4. Spine	Normal ☐ Abnormal ☐ Unknown ☐
	8.5. Abdomen	Normal ☐ Abnormal ☐ Unknown ☐
	8.6. Genitalia	Normal ☐ Abnormal ☐ Unknown ☐
	8.7. Other abnormalities	Yes ☐ No ☐
	8.7.1. If yes, describe	_____

If necessary, fill in the comments section

9	Does the child need admission to the hospital for any problem? *If the answer is yes please fill in an AE form*	Yes ☐ No ☐
10	10.1. Neuromuscular maturity	10.1.1 Posture score |__| 10.1.2 Square window |__| 10.1.3 Arm recoil |__| 10.1.4 Popliteal angle |__| 10.1.5 Scarf sign |__| 10.1.6 Heel to ear |__|
	Ballard test: 10.2. Physical maturity	10.2.1 Skin |__| 10.2.2 Lanugo |__| 10.2.3 Plantar surfasse |__| 10.2.4 Breast |__| 10.2.5 Eye-Ear |__| 10.2.6 Geniyals |__|
	THROAT SWAB **IF THE MOTHER'S PCR HAS BEEN POSITIVE AT PREGNANCY, PLEASE FILL IN THE FOLLOWING QUESTIONS**	
11	Was a throab swab for COVID-19 collected from the newborn?	Yes ☐ No ☐
12	IF yes, indicate the SARS-CoV-2 PCR result If positive, fill/update the Adverse Event Form	Positive ☐ Negative ☐
13	Was a rapid antigen test for COVID-19 performed?	Yes ☐ No ☐
14	IF yes, indicate the COVID-19 rapid antigen test result If Positive, fill/update the Adverse Event Form	Positive ☐ Negative ☐
	HIV PROPHYLAXIS **IF THE MOTHER TESTED POSITIVE FOR HIV**	
15	Has the newborn been given an ARV drug for HIV prophylaxis? *If yes, please fill out the Medication Form*	Yes ☐ No ☐

COMMENTS (OPTIONAL)

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7 _____

8 _____

BIRTH

Newborn laboratory results

MA-CoV **Event: Newborn laboratory results**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

	SARS-CoV-2 PCR LAB RESULTS (baseline) – *if a nasopharyngeal swab was collected*	
1	Date of the sample	|__|__|- |__|__|__|-|__|__|__|__| Day Month Year
2	SARS-CoV-2 PCR test result *If positive, fill/update the Adverse Event Form*	Positive ☐ Negative ☐
3	Ct value	|__|__|
4	SARS-CoV-2 Viral load	|__|__|__|__|__|__| copies/mL

COMMENTS (OPTIONAL)

1 _____

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3 _____

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POST-PARTUM VISIT

1 month after birth

Newborn questionnaire

MA-CoV **Event: Newborn laboratory results**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

	ATTENDANCE TO SCHEDULED VISIT	
1	Did the infant attend the scheduled visit to the health facility?	Yes ☐ No ☐
	1.1 *If the answer is no, please specify*	Migration ☐ Not found ☐ Absent ☐ Death ☐ Refused ☐ Other ☐ _____
	MEDICAL HISTORY AND PHYSICAL EXAMINATION	
2	Weight (g)	|__|__|__|__|__|
3	Length (cm)	|__|__|.|__|
4	MUAC (cm)	|__|__|.|__|
5	Head circumference (cm)	|__|__|.|__|
6	Axillary temperature (ºC)	|__|__|.|__|
7	Does the infant have a congenital abnormality not previously diagnosed?	Yes ☐ No ☐
8	Has the infant been admitted to the hospital since the last visit?	Yes ☐ No ☐
	COVID INFECTION SUSPICION **INQUIRY OF SIGNS AND SYMPTOMS DURING THE FIRST MONTH OF LIFE**	
9	Cough?	Yes ☐ No ☐
10	Fever? (Tª ≥ 37,5 ºC)	Yes ☐ No ☐
11	Shortness of breath?	Yes ☐ No ☐
12	Rhinorrhea?	Yes ☐ No ☐
13	Does the participant's legal guardian report fever during the last 24 hours?	Yes ☐ No ☐
	If Temp ≥ 37,5 ºC or the answer is YES for any of the questions, collect a throat swab for COVID-19	
14	Was a throat swab for COVID-19 collected? *If yes, please complete SARS-CoV-2 PCR lab results in the Laboratory Results Form*	Yes ☐ No ☐
15	Was a rapid antigen test for COVID-19 performed?	Yes ☐ No ☐
16	If yes, indicate the COVID-19 rapid antigen test result *If Positive, fill/update the Adverse Event Form*	Positive ☐ Negative ☐

	NUTRITION	
	Is the woman breastfeeding the infant?	Yes ☐ No ☐
17	17.1 If yes, please specify when breastfeeding started	Less than an hour after birth ☐ Between 1 and 12 hours after birth ☐ Between 12 and 24 hours after birth ☐ More than 24 hours after birth ☐
	During the first month of life, did the infant receive other foods or beverages apart from breast milk?	Yes ☐ No ☐
18	18.1 If yes, please specify which foods or beverages he/she received	*Water* ☐ *Juice* ☐ Other type of milk ☐ *Vegetables* ☐ *Fruit* ☐ *Sweets or sugar* ☐ *Traditional herbs* ☐ *Rice or cereals* ☐ *Other* ☐ Please specify: _____
	Yesterday, did the infant receive other foods or beverages apart from breast milk?	Yes ☐ No ☐
19	19.1 If yes, please specify which foods or beverages he/she received	*Water* ☐ *Juice* ☐ Other type of milk ☐ *Vegetables* ☐ *Fruit* ☐ *Sweets or sugar* ☐ *Traditional herbs* ☐ *Rice or cereals* ☐ *Other* ☐ Please specify: _____
	PSYCHOMOTOR DEVELOPMENT ASSESSMENT	
20	Was the psychomotor development assessed?	Yes ☐ No ☐
	Gross motor skills	
21	21.1 Does the infant move the 4 extremities symmetrically?	Yes ☐ No ☐
	21.2 Muscle tone	Normal ☐ Abnormal ☐
	Fine motor skills	
22	Does the infant follow objects?	Yes ☐ No ☐
	Language / audition	
23	Does the infant respond to sounds?	Yes ☐ No ☐
	Social skills	
24	Does the infant respond to smiles?	Yes ☐ No ☐

	COMMENTS (OPTIONAL)
1	_____
2	_____
3	_____
4	_____

POST-PARTUM

1 month after birth

Newborn laboratory results

MA-CoV NCT03671109 **Event: Laboratory results Newborn**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

	SARS-CoV-2 PCR LAB RESULTS (1 month after birth)	
1	Date of the sample	|__|__|- |__|__|__|-|__|__|__|__| Day Month Year
2	SARS-CoV-2 PCR test result *If positive, fill/update the Adverse Event Form*	Positive ☐ Negative ☐
3	Ct value	|__|__|
4	SARS-CoV-2 Viral load	|__|__|__|__|__|__| copies/mL
	HIV PCR LAB RESULTS – *if a HIV PCR was done*	
5	Was an HIV PCR done?	Yes ☐ No ☐
5.1	*If yes,* Date of the sample	|__|__|- |__|__|__|-|__|__|__|__|
5.2	HIV PCR test result *If positive, fill/update the Adverse Event Form*	Positive ☐ Negative ☐

COMMENTS (OPTIONAL)

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STUDY COMPLETION FORM

STUDY COMPLETION FORM MA-CoV **Event: Study completion form Newborn**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

	STUDY COMPLETION	
1	Date of last contact?	|__|__| - |__|__|__| - |__|__|__|__| Day Month Year
2	Did the newborn complete the study? *2.1 If the answer is no, please provide all relevant information related to reason for premature discontinuation*	Yes ☐ No ☐ Death ☐ Serious health outcome ☐ Consent withdrawal ☐ Migration ☐ Lost to follow up ☐ Other ☐ Specify: _____
3	Date of participant's study completion	|__|__| - |__|__|__| - |__|__|__|__| Day Month Year
4	I have reviewed and found all data pertaining to this participant to be complete and accurate Printed Investigator's name _____	|__|__| - |__|__|__| - |__|__|__|__| Day Month Year

Please provide all relevant information related to reason for premature study discontinuation including contributory factors in the comments section

COMMENTS (OPTIONAL)

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ADVERSE EVENTS FORM

MA-CoV	**ID** MACOC|__|-|__|__|__|__|__| Site Code Subject Nº	**Participant's initials** |__|__| |__| 1. 2. Family name	

Severity: **1** Mild (Grade 1): Awareness of sign or symptom easily tolerated, **2** Moderate (Grade 2): Discomfort enough to cause interference with usual activity, **3** Severe (Grade 3): Incapacitating with inability to work or perform usual activity, **4** Life-threatening (Grade 4): Patient at risk of death at the time of the event, or Event Results in death, or Requires hospitalization or prolongation of existing hospitalization, or Results in persistent or significant disability or incapacity or Consists of a congenital anomaly or birth defect

Outcome: 1-Completely recovered, 2-Not yet completely recovered, 3-Deterioration, 4-Permanent damage, 5-Death, 6-Ongoing, 7-Unknown

Action Taken: 1-No action taken, , 2-Concomitant medication given, 3-Non-drug therapy given, 4-Hospitalization/Hospitalization prolonged

ADVERSE EVENT #

1	A. Name / Description |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|	B. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	C. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Month Year
2	A. Severity ☐ 1 ☐ 2 ☐ 3 ☐ 4	B. Outcome ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7	C. Action taken ☐ 1 ☐ 2 ☐ 3 ☐ 4
3	Is this AE Serious? ☐NO ☐YES		

ADVERSE EVENT #

1	A. Name / Description |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|	B. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	C. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Month Year
2	A. Severity ☐ 1 ☐ 2 ☐ 3 ☐ 4	B. Outcome ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7	C. Action taken ☐ 1 ☐ 2 ☐ 3 ☐ 4
3	Is this AE Serious? ☐NO ☐YES		

ADVERSE EVENT #

1	A. Name / Description |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|	B. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	C. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Month Year
2	A. Severity ☐ 1 ☐ 2 ☐ 3 ☐ 4	B. Outcome ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7	C. Action taken ☐ 1 ☐ 2 ☐ 3 ☐ 4
3	Is this AE Serious? ☐NO ☐YES		

MEDICATION FORMS

MA-CoV Medication forms	ID MACOC|__|-|__|__|__|__| Site Code Subject Nº	Participant's initials |__|__| |__| 1. 2. Family name	

	Medication #1			
1	A. Medication/ Non-drug therapy |__|__|__|__|__|__|__|__|__|	B. Dosage |__|__|__|__|__|__|	C. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	D. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Day Month Year
2	A. Reason to take it _____	B. Is an AE the cause of taking it? ☐YES (fill an AE form) ☐NO, the cause started prior to recruitment		

	Medication #2			
1	A. Medication/ Non-drug therapy |__|__|__|__|__|__|__|__|__|	B. Dosage |__|__|__|__|__|__|	C. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	D. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Day Month Year
2	A. Reason to take it _____	B. Is an AE the cause of taking it? ☐YES (fill an AE form) ☐NO, the cause started prior to recruitment		

	Medication #3			
1	Medication/ Non-drug therapy |__|__|__|__|__|__|__|__|__|	B. Dosage |__|__|__|__|__|__|	C. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	D. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Day Month Year
2	A. Reason to take it _____	B. Is an AE the cause of taking it? ☐YES (fill an AE form) ☐NO, the cause started prior to recruitment		

	Medication #4			
1	A. Medication/ Non-drug therapy |__|__|__|__|__|__|__|__|__|	B. Dosage |__|__|__|__|__|__|	C. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	D. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Day Month Year
2	A. Reason to take it _____	B. Is an AE the cause of taking it? ☐YES (fill an AE form) ☐NO, the cause started prior to recruitment		

ISGlobal Barcelona Institute for Global Health

MA-COV Maternal health and COVID-19

ID MACOC- |__|-|__|__|__|__|
Site Code Subject Number

Participant's initials |__|__| |__|
1. 2. Family name

Prevalence and impact of SARS-CoV-2 infection on maternal and infant health in African populations (MA-CoV)

Project Acronym	MA-CoV

eCRF book ID MA-CoV
Version: v.3.1
Date: 28th April 2022

BIRTH

Newborn visit

Ma-COV **Event: Birth - Newborn visit**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

	INCLUSION CRITERIA CHECK	
1	Mother's ID	MACOW|__|-|__|__|__|__| Site Code Subject Nº
2	Date of birth	|__|__| - |__|__|__| - |__|__|__|__| Day Month Year
3	Sex	Masculine ☐ Feminine ☐
	MEDICAL HISTORY AND PHYSICAL EXAMINATION AT BIRTH	
4	Weight (g)	|__|__|__|__|__|
5	Length (cm)	|__|__|.|__|
6	Head circumference (cm)	|__|__|.|__|
7	Axillary temperature (ºC)	|__|__|.|__|
8	Congenital abnormalities?	Yes ☐ No ☐
	8.1. Face and head	Normal ☐ Abnormal ☐ Unknown ☐
	8.2. Limbs	Normal ☐ Abnormal ☐ Unknown ☐
	8.3. Chest	Normal ☐ Abnormal ☐ Unknown ☐
	8.4. Spine	Normal ☐ Abnormal ☐ Unknown ☐
	8.5. Abdomen	Normal ☐ Abnormal ☐ Unknown ☐
	8.6. Genitalia	Normal ☐ Abnormal ☐ Unknown ☐
	8.7. Other abnormalities	Yes ☐ No ☐
	8.7.1. If yes, describe	_____

If necessary, fill in the comments section

9	Does the child need admission to the hospital for any problem? *If the answer is yes please fill in an AE form*	Yes ☐ No ☐
10	10.1. Neuromuscular maturity	10.1.1 Posture score |__| 10.1.2 Square window |__| 10.1.3 Arm recoil |__| 10.1.4 Popliteal angle |__| 10.1.5 Scarf sign |__| 10.1.6 Heel to ear |__|
	Ballard test: 10.2. Physical maturity	10.2.1 Skin |__| 10.2.2 Lanugo |__| 10.2.3 Plantar surfasse |__| 10.2.4 Breast |__| 10.2.5 Eye-Ear |__| 10.2.6 Geniyals |__|
	THROAT SWAB **IF THE MOTHER'S PCR HAS BEEN POSITIVE AT PREGNANCY, PLEASE FILL IN THE FOLLOWING QUESTIONS**	
11	Was a throab swab for COVID-19 collected from the newborn?	Yes ☐ No ☐
12	IF yes, indicate the SARS-CoV-2 PCR result If positive, fill/update the Adverse Event Form	Positive ☐ Negative ☐
13	Was a rapid antigen test for COVID-19 performed?	Yes ☐ No ☐
14	IF yes, indicate the COVID-19 rapid antigen test result If Positive, fill/update the Adverse Event Form	Positive ☐ Negative ☐
	HIV PROPHYLAXIS **IF THE MOTHER TESTED POSITIVE FOR HIV**	
15	Has the newborn been given an ARV drug for HIV prophylaxis? *If yes, please fill out the Medication Form*	Yes ☐ No ☐

COMMENTS (OPTIONAL)

1 _____

2 _____

3 _____

4 _____

5 _____

6 _____

7 _____

8 _____

BIRTH

Newborn laboratory results

MA-CoV **Event: Newborn laboratory results**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

SARS-CoV-2 PCR LAB RESULTS (baseline) – *if a nasopharyngeal swab was collected*		
1	Date of the sample	|__|__|- |__|__|__|-|__|__|__|__| Day Month Year
2	SARS-CoV-2 PCR test result *If positive, fill/update the Adverse Event Form*	Positive ☐ Negative ☐
3	Ct value	|__|__|
4	SARS-CoV-2 Viral load	|__|__|__|__|__|__| copies/mL

COMMENTS (OPTIONAL)

1 _____

2 _____

3 _____

4 _____

5 _____

6 _____

7 _____

8 _____

POST-PARTUM VISIT

1 month after birth

Newborn questionnaire

MA-CoV **Event: Newborn laboratory results**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

	ATTENDANCE TO SCHEDULED VISIT	
1	Did the infant attend the scheduled visit to the health facility?	Yes ☐ No ☐
	1.1 *If the answer is no, please specify*	Migration ☐ Not found ☐ Absent ☐ Death ☐ Refused ☐ Other ☐ _____
	MEDICAL HISTORY AND PHYSICAL EXAMINATION	
2	Weight (g)	|__|__|__|__|__|
3	Length (cm)	|__|__|.|__|
4	MUAC (cm)	|__|__|.|__|
5	Head circumference (cm)	|__|__|.|__|
6	Axillary temperature (ºC)	|__|__|.|__|
7	Does the infant have a congenital abnormality not previously diagnosed?	Yes ☐ No ☐
8	Has the infant been admitted to the hospital since the last visit?	Yes ☐ No ☐
	COVID INFECTION SUSPICION **INQUIRY OF SIGNS AND SYMPTOMS DURING THE FIRST MONTH OF LIFE**	
9	Cough?	Yes ☐ No ☐
10	Fever? ($T^a \geq 37,5$ ºC)	Yes ☐ No ☐
11	Shortness of breath?	Yes ☐ No ☐
12	Rhinorrhea?	Yes ☐ No ☐
13	Does the participant's legal guardian report fever during the last 24 hours?	Yes ☐ No ☐
	If Temp ≥ 37,5 ºC or the answer is YES for any of the questions, collect a throat swab for COVID-19	
14	Was a throat swab for COVID-19 collected? *If yes, please complete SARS-CoV-2 PCR lab results in the Laboratory Results Form*	Yes ☐ No ☐
15	Was a rapid antigen test for COVID-19 performed?	Yes ☐ No ☐
16	If yes, indicate the COVID-19 rapid antigen test result *If Positive, fill/update the Adverse Event Form*	Positive ☐ Negative ☐

	NUTRITION	
	Is the woman breastfeeding the infant?	Yes ☐ No ☐
17	17.1 If yes, please specify when breastfeeding started	Less than an hour after birth ☐ Between 1 and 12 hours after birth ☐ Between 12 and 24 hours after birth ☐ More than 24 hours after birth ☐
	During the first month of life, did the infant receive other foods or beverages apart from breast milk?	Yes ☐ No ☐
18	18.1 If yes, please specify which foods or beverages he/she received	*Water* ☐ *Juice* ☐ Other type of milk ☐ *Vegetables* ☐ *Fruit* ☐ *Sweets or sugar* ☐ *Traditional herbs* ☐ *Rice or cereals* ☐ *Other* ☐ Please specify: _____
	Yesterday, did the infant receive other foods or beverages apart from breast milk?	Yes ☐ No ☐
19	19.1 If yes, please specify which foods or beverages he/she received	*Water* ☐ *Juice* ☐ Other type of milk ☐ *Vegetables* ☐ *Fruit* ☐ *Sweets or sugar* ☐ *Traditional herbs* ☐ *Rice or cereals* ☐ *Other* ☐ Please specify: _____
	PSYCHOMOTOR DEVELOPMENT ASSESSMENT	
20	Was the psychomotor development assessed?	Yes ☐ No ☐
	Gross motor skills	
21	21.1 Does the infant move the 4 extremities symmetrically?	Yes ☐ No ☐
	21.2 Muscle tone	Normal ☐ Abnormal ☐
	Fine motor skills	
22	Does the infant follow objects?	Yes ☐ No ☐
	Language / audition	
23	Does the infant respond to sounds?	Yes ☐ No ☐
	Social skills	
24	Does the infant respond to smiles?	Yes ☐ No ☐

COMMENTS (OPTIONAL)

1 _____

2 _____

3 _____

4 _____

POST-PARTUM

1 month after birth

Newborn laboratory results

MA-CoV NCT03671109 **Event: Laboratory results Newborn**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

	SARS-CoV-2 PCR LAB RESULTS (1 month after birth)	
1	Date of the sample	|__|__|- |__|__|__|-|__|__|__|__| Day Month Year
2	SARS-CoV-2 PCR test result *If positive, fill/update the Adverse Event Form*	Positive ☐ Negative ☐
3	Ct value	|__|__|
4	SARS-CoV-2 Viral load	|__|__|__|__|__|__| copies/mL
	HIV PCR LAB RESULTS – *if a HIV PCR was done*	
5	Was an HIV PCR done?	Yes ☐ No ☐
5.1	*If yes,* Date of the sample	|__|__|- |__|__|__|-|__|__|__|__|
5.2	HIV PCR test result *If positive, fill/update the Adverse Event Form*	Positive ☐ Negative ☐

COMMENTS (OPTIONAL)

1 _____

2 _____

3 _____

4 _____

5 _____

6 _____

7 _____

8 _____

STUDY COMPLETION FORM

STUDY COMPLETION FORM MA-CoV **Event: Study completion form Newborn**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

	STUDY COMPLETION	
1	Date of last contact?	|__|__| - |__|__|__| - |__|__|__|__| Day Month Year
2	Did the newborn complete the study?	Yes ☐ No ☐
	2.1 If the answer is no, please provide all relevant information related to reason for premature discontinuation	Death ☐ Serious health outcome ☐ Consent withdrawal ☐ Migration ☐ Lost to follow up ☐ Other ☐ Specify: _____
3	Date of participant's study completion	|__|__| - |__|__|__| - |__|__|__|__| Day Month Year
4	I have reviewed and found all data pertaining to this participant to be complete and accurate	
	Printed Investigator's name _____	|__|__| - |__|__|__| - |__|__|__|__| Day Month Year

Please provide all relevant information related to reason for premature study discontinuation including contributory factors in the comments section

COMMENTS (OPTIONAL)

1 _____

2 _____

3 _____

4 _____

5 _____

6 _____

7 _____

8 _____

9 _____

10 _____

ADVERSE EVENTS FORM

MA-CoV	ID MACOC|__|-|__|__|__|__| Site Code Subject Nº	Participant's initials |__|__| |__| 1. 2. Family name	

Severity: **1** Mild (Grade 1): Awareness of sign or symptom easily tolerated, **2** Moderate (Grade 2): Discomfort enough to cause interference with usual activity, **3** Severe (Grade 3): Incapacitating with inability to work or perform usual activity, **4** Life-threatening (Grade 4): Patient at risk of death at the time of the event, or Event Results in death, or Requires hospitalization or prolongation of existing hospitalization, or Results in persistent or significant disability or incapacity or Consists of a congenital anomaly or birth defect

Outcome: 1-Completely recovered, 2-Not yet completely recovered, 3-Deterioration, 4-Permanent damage, 5-Death, 6-Ongoing, 7-Unknown

Action Taken: 1-No action taken, , 2-Concomitant medication given, 3-Non-drug therapy given, 4-Hospitalization/Hospitalization prolonged

ADVERSE EVENT #

1	A. Name / Description |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|	B. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	C. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Month Year
2	A. Severity ☐ 1 ☐ 2 ☐ 3 ☐ 4	B. Outcome ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7	C. Action taken ☐ 1 ☐ 2 ☐ 3 ☐ 4
3	Is this AE Serious? ☐NO ☐YES		

ADVERSE EVENT #

1	A. Name / Description |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|	B. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	C. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Month Year
2	A. Severity ☐ 1 ☐ 2 ☐ 3 ☐ 4	B. Outcome ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7	C. Action taken ☐ 1 ☐ 2 ☐ 3 ☐ 4
3	Is this AE Serious? ☐NO ☐YES		

ADVERSE EVENT #

1	A. Name / Description |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|	B. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	C. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Month Year
2	A. Severity ☐ 1 ☐ 2 ☐ 3 ☐ 4	B. Outcome ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7	C. Action taken ☐ 1 ☐ 2 ☐ 3 ☐ 4
3	Is this AE Serious? ☐NO ☐YES		

MEDICATION FORMS

MA-CoV Medication forms	ID MACOC|__|-|__|__|__|__| Site Code Subject Nº	Participant's initials |__|__| |__| 1. 2. Family name	

Medication #1

	A. Medication/ Non-drug therapy	B. Dosage	C. Start date	D. End date
1	|__|__|__|__|__|__|__|__|__|	|__|__|__|__|__|__|	|__|__| |__|__|__||__|__|__|__| Day Month Year	|__|__| |__|__|__||__|__|__|__| ☐ Ongoing Day Month Year
2	A. Reason to take it _____	B. Is an AE the cause of taking it? ☐YES (fill an AE form) ☐NO, the cause started prior to recruitment		

Medication #2

	A. Medication/ Non-drug therapy	B. Dosage	C. Start date	D. End date
1	|__|__|__|__|__|__|__|__|__|	|__|__|__|__|__|__|	|__|__| |__|__|__||__|__|__|__| Day Month Year	|__|__| |__|__|__||__|__|__|__| ☐ Ongoing Day Month Year
2	A. Reason to take it _____	B. Is an AE the cause of taking it? ☐YES (fill an AE form) ☐NO, the cause started prior to recruitment		

Medication #3

	Medication/ Non-drug therapy	B. Dosage	C. Start date	D. End date
1	|__|__|__|__|__|__|__|__|__|	|__|__|__|__|__|__|	|__|__| |__|__|__||__|__|__|__| Day Month Year	|__|__| |__|__|__||__|__|__|__| ☐ Ongoing Day Month Year
2	A. Reason to take it _____	B. Is an AE the cause of taking it? ☐YES (fill an AE form) ☐NO, the cause started prior to recruitment		

Medication #4

	A. Medication/ Non-drug therapy	B. Dosage	C. Start date	D. End date
1	|__|__|__|__|__|__|__|__|__|	|__|__|__|__|__|__|	|__|__| |__|__|__||__|__|__|__| Day Month Year	|__|__| |__|__|__||__|__|__|__| ☐ Ongoing Day Month Year
2	A. Reason to take it _____	B. Is an AE the cause of taking it? ☐YES (fill an AE form) ☐NO, the cause started prior to recruitment		

BMJ Open

Prevalence and impact of SARS-CoV-2 infection on maternal and infant health in African populations: protocol of a multi-centre prospective cohort study (MA-CoV project)

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Secondary Subject Heading:	Epidemiology, Infectious diseases
Keywords:	COVID-19, Maternal medicine < OBSTETRICS, Epidemiology < INFECTIOUS DISEASES, HIV & AIDS < INFECTIOUS DISEASES

Prevalence and impact of SARS-CoV-2 infection on maternal and infant health in African populations: protocol of a multi-centre prospective cohort study (MA-CoV project)

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ABSTRACT

Introduction. Pregnant women are currently considered a vulnerable population to SARS-CoV-2 infection, with increased risk of severe COVID-19, pre-term birth and maternal mortality. There is, however, a paucity of data on the burden of maternal SARS-CoV-2 infection in sub-Saharan countries. The objective of this study is to determine the prevalence and health effects of maternal SARS-CoV-2 infection in selected sites from Gabon and Mozambique.

Methods and analysis. MA-CoV (MAternal CoVid) is an observational, multicenter, prospective cohort study where 1000 pregnant women (500 per country) will be enrolled at the antenatal clinic visits. Participants will undergo monthly follow-up at each antenatal care visit, delivery and post-partum visit. The primary study outcome is the prevalence of SARS-CoV-2 infection during pregnancy. The clinical presentation of COVID-19 in pregnancy will also be characterized, and incidence of infection during pregnancy will be evaluated, as well as the risk factors of maternal and neonatal morbidity and mortality associated with SARS-CoV-2 infection and the risk of mother-to-child transmission of SARS-CoV-2. SARS-CoV-2 infection screening will be performed through polymerase chain reaction (PCR) diagnosis.

Ethics and dissemination. The protocol was reviewed and approved by the *Comité National d'Éthique pour la Recherche au Gabon*, *Comité Nacional de Bioética para Saúde de Moçambique* and the Ethics Committee of the Hospital Clinic of Barcelona (Spain). Project results will be presented to all stakeholders and published in open-access journals.

Registration number. NCT05303168.

Strengths and limitations

- The prospective longitudinal study design which covers the pregnancy and post-partum periods will allow us to assess both prevalence and incidence of SARS-CoV-2 infection and its health effects in pregnancy and perinatal outcomes.
- The inclusion of participants from two different sub-Saharan countries will provide information on the differences in the distribution of SARS-CoV-2 infection.
- The COVID-19 pandemic scenario presents several barriers and challenges not only due to the disease itself but also due to the implementation of containment measures such as quarantine, social distancing and community containment. This may affect antenatal care attendance, as well as hinder the implementation of the study.
- As COVID-19 vaccination of study participants may limit our capacity to estimate the prevalence of the infection through SARS-CoV-2 serology, we will assess the prevalence of antibodies against the SARS-CoV-2 nucleocapsid protein, which reflects past SARS-CoV-2 infection.

Keywords: SARS-CoV-2, sub-Saharan Africa, pregnancy

INTRODUCTION

As of July 2022, more than nine million COVID-19 cases and 170,000 related deaths have been reported in the World Health Organization (WHO) African region, while only 21% of the African population has been fully vaccinated.[1] However, the real burden of SARS-CoV-2 in Africa is probably still unknown and underestimated.

Pregnant women are at increased susceptibility of SARS-CoV-2 infection, particularly those with co-morbidities such as preeclampsia and gestational diabetes mellitus.[2] This may be explained by the pregnancy-induced changes, which include a decreased lung volume, an increased risk for thromboembolic disease and immunological changes in order to allow for the growth of a semi-allogenic fetus.[3] Effects of SARS-CoV-2 infection on maternal and neonatal health include increased risk of admission to intensive care and need of mechanical ventilation, induced abortion, c-section, pre-term birth, foetal growth restriction, post-partum hemorrhage and maternal mortality. [4-6]

Besides, mother to child transmission of SARS-CoV-2 is possible intrauterine, intrapartum, and at the postpartum period.[7] Several studies have reported the detection of SARS-CoV-2 in the fetal side of the placenta, indicating transplacental foetal infection.[7] Of note, most of the evidence of these effect has been gathered in high income countries.

In Sub-Saharan Africa (SSA), SARS-CoV-2 overlaps geographically with endemic infectious diseases such as the Human Immunodeficiency Virus (HIV) and malaria in a context of low SARS-CoV-2 vaccination coverage. For instance, co-infection with SARS-CoV-2 and malaria in pregnant women, might have deleterious effects in the foetal development, considering the reported inflammatory and histologic changes at the placental level found in both infections.[8 9] Additionally, there is evidence that immunosuppressed HIV-infected individuals are at increased risk of severe COVID-19 and death than non-infected individuals.[10 11] Importantly, the burden of HIV infection is concentrated in SSA.[12]

The information on the burden of SARS-CoV-2 infection in pregnancy in SSA countries is very limited. As to date, most of studies have been carried out in high income countries, neglecting the particular characteristics of SARS-CoV-2 infection in pregnancy in low-middle income countries. In this context, the present study was developed leveraging on an ongoing multicenter, two-arm, placebo-controlled, individually randomized trial aiming to assess the efficacy and safety of dihydroartemisinin-piperaquine as intermittent preventive treatment for HIV-infected pregnant women (NCT03671109).[13]

Study aims and hypotheses

The primary objective of the MA-CoV (Maternal CoVid) study is to determine the prevalence and incidence of SARS-CoV-2 infection during pregnancy. Secondary objectives include to describe the effects of maternal SARS-CoV-2 infection on pregnancy and perinatal outcomes, to characterize the clinical features of COVID-19 disease in pregnancy, and to assess the potential vertical transmission and through breastfeeding of SARS-CoV-2 from infected mothers to their offspring. The main study hypotheses are: (1) SARS-CoV-2 infection during pregnancy may influence maternal and perinatal outcomes, (2) SARS-CoV-2 clinical manifestations may be different in pregnant women compared to non-pregnant adults, and (3) SARS-CoV-2 can be transmitted from mother to child prenatally and postnatally.

METHODS AND ANALYSIS

MA-CoV is an observational, multicenter prospective cohort study.

Study settings

The study will be carried out in Libreville and Lambaréné (Gabon), and in Manhiça (Mozambique). SARS-CoV-2 reported cases ranged from 48,000 in Gabon to 228,000 in Mozambique as per July 2022.[14] Additionally, HIV prevalence among pregnant women ranges from 6% in study sites of Gabon to 29% in study sites of Mozambique.[15] [16] Malaria epidemiological indicators and SARS-CoV-2 and HIV prevalence in pregnancy in study sites are shown in table 1.

Table 1. SARS-CoV-2, malaria and HIV epidemiology in study countries

Country	Site	SARS-CoV-2 reported cases (country-level)	*P.falciparum* infection prevalence in women at delivery†	HIV prevalence in pregnant women
Mozambique	Manhiça	228,000[1]	6%	29%[15]
Gabon	Lambaréné	48,000[1]	11%	6%[16]
	Libreville		NI	6%[16]

†Data from 2010-2012 in women receiving either two IPTp doses of mefloquine or SP (Tuikue-Ndam et al, unpublished). NI: No information; MTCT: mother-to-child transmission

Study population

All pregnant women attending the study antenatal care (ANC) services will be screened for participation in the study. Inclusion criteria are: (1) permanent resident in the study area and (2) willing to deliver in the study maternity wards. Pregnant women planning to move out the study area in the following 7 months from enrolment will be excluded.

Informed consent and recruitment

All participants will receive information about study procedures. A signed informed consent form (or thumb-printed with a witness if the woman is illiterate) will be obtained before any study procedures are carried out by study nurses in each site. The informed consent will cover the woman and the new born infant. The study's informed consent is available as Supplementary Material file 1. If the participant is under the legal age of maturity, she will sign the assent form and her legal guardian will sign the informed consent according to national ethics local policies.

After the study details are explained and informed consent is signed, a study identification card containing the individual study number and basic demographic information will be given to the participant in order to facilitate identification at all study contacts.

Follow-up and measurement of outcomes

At baseline, the woman's demographic and obstetric information will be recorded in study specific case report forms (CRFs) (Supplementary material file 2)

Physical and clinical examination at enrolment

The physical examination of the woman will include the following assessments: weight, height, gestational age by bimanual palpation and measurement of middle-upper arm circumference (MUAC). Ultrasound will be performed to determine gestational age and confirm pregnancy viability at enrolment if possible. COVID-19 suggestive symptoms will be assessed, and should

the woman present them, A nasopharyngeal swab will be collected for detection of SARS-CoV-2 viral RNA. Additionally, a nasopharyngeal swab will be collected in a sub-sample of 100 study participants regardless presence of COVID-19 symptoms for screening of SARS-CoV-2 infection.

Baseline biological samples

At enrolment, a venous blood sample (5 mL) will be collected for analysis of hemoglobin level, SARS-CoV-2 total antibodies, malaria polymerase chain reaction (PCR) (if the woman presents malaria-suggestive symptoms), and HIV viral load and CD4 cell count (if the woman is HIV-infected).

Antenatal follow-up

Participants will receive the standard ANC package of interventions, which includes intermittent preventive treatment (IPTp) of malaria, iron and folate supplementation, following national guidelines. During monthly ANC visits. COVID-19 suggestive symptoms will be assessed, and should the woman present them, a PCR to detect SARS-CoV-2 viral load will be performed.

Unscheduled visits

Study participants reporting being sick at the health facilities (including suspicion of COVID-19) will be seen by study personnel. Every unscheduled visit of the woman from enrolment until the post-partum visit will be recorded into a study CRF.

End of pregnancy and post-partum period

At the end of pregnancy, 5 ml of maternal blood sample will be collected for analysis of antibodies (IgG and IgM) against SARS-CoV-2, malaria parasitaemia and HIV viral load (in case the woman is HIV-infected). Additionally, whenever possible, cord blood and placental tissue samples will be collected for SARS-CoV-2 serologic and PCR analysis, respectively.

Breastmilk samples (3 ml) will also be collected within the first three days after delivery (colostrum) and at the post-partum visit (approximately six weeks after the end of pregnancy), for detection of SARS-CoV-2 by PCR. In addition, a neonatal throat swab will be collected at birth for SARS-CoV-2 analysis by PCR in infants born to COVID-19 positive mothers. A summary of study procedures is displayed in Table 2.

Table 2. Study visits and procedures schedule

Study procedure	First ANC clinic visit	Routine ANC clinic visits	End of pregnancy	1 month after end of pregnancy	Unscheduled visits	Infant Assessment (birth and 1 month)
Inclusion/ Exclusion criteria check	X					
Written informed consent	X					
Demographics, socio-economic/ Medical history	X				X	
COVID-19 screening#	X	X			X	X
Record of medications/ Morbidity	X	X	X	X	X	
Physical examination/clinical	X		X		X	
Gestational age	X	X	X		X	
Temperature				X	X	X
Blood Pressure	X		X	X	X	
Weight	X	X		X	X	
Height	X					
MUAC	X			X		
Presence of proteins in urine	X					
CD4 count*	X					
HIV viral load*	X		X			
SARS-CoV-2 serology	X		X			
Malaria blood PCR	X					
Blood smear	†	†	X	X	†	
Haemoglobin test	X		X	X		
Peripheral venous blood (mother)	X		X			
Cord blood			X			
Placental biopsy			X			
Placental impression smears			X			
Breastmilk (SARS-CoV2)			X	X		

ANC: Antenatal care; MUAC: middle-upper circumference

In participants with suggestive symptoms/signs of COVID-19 (fever, cough, shortness of breath, sudden onset of anosmia, ageusia or dysgeusia), except in a sub-sample of 100 participants at enrolment among whom it will be performed regardless of presence of symptoms.

*Only in HIV-infected women

† Only in women passively reporting sick AND presenting with malaria related signs/symptoms (fever (≥37,5º C) or having history of fever in the past 24 hours, arthromyalgia or headache), as per national management guidelines

Infant assessment

A neonatal throat swab will be collected at birth for SARS-CoV-2 analysis by PCR in infants born to COVID-19 positive mothers. Should the neonate present with symptoms and/or signs suggestive of acute respiratory infection during the first month of life, another throat swab will be collected for SARS-CoV-2 testing by PCR.

Laboratory tests

Detection of SARS-CoV-2

A real-time polymerase chain reaction (RT-PCR) COVID-19 assay diagnostic test will be performed at the study laboratories for detection of SARS-CoV-2 viral RNA. Real-Time PCR technology utilizes polymerase chain reaction for the amplification of specific target sequences and target specific probes for the detection of the amplified RNA. The probes are labelled with fluorescent reporter and quencher dyes.

The Elecsys® Anti-SARS-CoV-2 and Elecsys® Anti-SARS-CoV-2 S essays (Roche Diagnostics) will be used for detection of total anti-SARS-CoV-2 spike (S) and nucleocapsid (N) antibodies through electro-chemiluminescent immunoassays (ECLIA) intended for the qualitative detection of total antibodies (including IgG and IgM) to SARS-CoV-2 in human serum and plasma.[17 18] This assay is a double-antigen sandwich electrochemiluminescence immunoassay, which separates bound from unbound substances with streptavidin-coated microparticles before applying a voltage to the electrode.[17 18]

Malaria parasitological and haematological determinations

In case of malaria suspicion, thick and thin blood smears will be collected and stained with Giemsa's stain and examined for *Plasmodium spp*. following standard procedures. Also, blood haemoglobin will be determined following local SOPs.

Detection of HIV and quantitative determination of viral load

In HIV-infected women, quantitative PCR HIV viral load will be determined from the venous blood samples drawn at enrolment and at delivery. HIV viral load will be determined from plasma cryopreserved at -80ºC using the devices in place *in the study sites (such as* COBAS® AMPLICOR, AmpliPrep [Roche Diagnostics] *or GeneXpert).*

Immunological determinations related to HIV status

In HIV-infected women, CD4+T cell count will be determined by flow cytometry after staining of whole blood with CD3, CD8 and CD4 fluorochrometolabelled antibodies and acquisition using FACSCalibur (BD Biosciences) and TruCOUNT tubes (Becton Dickinson, San Jose, CA; USA) *or MiniVIdas device*.

Placental samples analysis

A placental sample will be collected for malaria histological analysis. The biopsies will be immediately placed in 25 mL of 10% neutral buffered formalin and kept at 4ºC until processed and embedded in paraffin wax by standard techniques. Paraffin sections will be stained with haematoxylin and eosin, Giemsa's stain and the periodic acid-Schiff technique. Placental histology will include the examination of inflammatory signs (such as presence of neutrophils and monocytes) in the subchorial space and the umbilical cord connective tissue (funisitis) and analysis of intervillous fibrin deposition.[19]

Additionally, another placental sample will be collected for SARS-CoV-2 histopathological detection. The placental tissue will be placed in a sterile 150 ml bottle and kept in a -80ºC freezer until the sample is processed. Placental histology will include the examination of inflammatory signs (such as presence of neutrophils and monocytes) in the subchorial space and the umbilical cord connective tissue (funisitis) and analysis of intervillous fibrin deposition.[19]

Data management

All the data will be collected using paper CRFs during the study visits, from interviews and clinical observation or measures taken to participants. Results from the laboratory analyses performed in collected participant's biological samples will also be collected and entered in the CRFs.

Data from the study source document will be double entered into the study database using the OpenClinica open source software version 3.1.4 (Copyright OpenClinica LLC and collaborators, Waltham, MA, USA, www.OpenClinica.com) Subsequently, entered data will be systematically checked by Data Management team using error messages printed from validation programs and database listings. Quality control audits of all key safety and efficacy information in the database will be made prior to locking the database.

Study outcomes

The primary outcome of the study will be the prevalence of anti-SARS-CoV-2 N protein total antibodies at delivery. The secondary endpoints can be found in Table 3.

Table 3. Study endpoints

Primary endpoint	Prevalence of anti-SARS-CoV-2 N protein antibodies (Ig G and/or Ig M positive) against SARS-CoV-2 among pregnant women at delivery
Secondary endpoints	1. PCR-confirmed SARS-CoV-2 infection among pregnant women at recruitment 2. Incidence of SARS-CoV-2 infection during pregnancy 3. Maternal and neonatal morbidity and mortality due to SARS-CoV-2 infection during pregnancy 4. Pregnancy and perinatal adverse outcomes 5. Rate of vertical transmission of SARS-CoV-2 from infected mothers to their offspring, during the prenatal and perinatal period 6. CD4 cell counts and HIV viral load 7. Malaria parasitaemia at delivery (from maternal sample collected at delivery)

Sample size

Considering six months of enrolment and recruitment rates of participants in ongoing clinical trials in the two study sites, it was expected to include approximately 1000 women in the study. Assuming a 5% prevalence of SARS-CoV-2 of infection during pregnancy, this sample size would allow estimating the proportion of women with the infection with a 1.4% precision at the 95% confidence level.[20]

Statistical analysis

Infection by SARS-CoV-2 will be defined by presence of anti-SARS-CoV-2 nucleocapsid (N) protein antibodies (total IgG, IgG and/or IgM) or by a positive SARS-CoV-2 PCR. Asymptomatic COVID-19 infection will be defined by presence of SARS-CoV-2 nucleocapsid (N) antibodies and/or a positive COVID-19 PCR without COVID-19 associated symptoms. Women with baseline anti-SARS-CoV-2 N protein SARS-CoV-2 antibodies will be considered infected before study enrolment.

The socio-demographic characteristics of the study participants will be described using summary statistics. Continuous variables will be summarized using mean or median (depending on the

distribution of the variable) and standard deviation or interquartile range. Categorical variables will be described using frequencies and percentages. Proportions for categorical variables will be assessed using the chi-square test or Fisher's exact test where appropriate. The Student's t test or Wilcoxon rank-sum test will be used to compare means and medians, respectively, of continuous variables according to variable characteristics. Only records with information on the outcome of interest will be analyzed.

Incidences of all-cause hospital admissions and all-cause outpatient attendance during pregnancy will be analyzed using negative binomial regression and compared by SARS-CoV-2 infection status. The incidence of COVID-19 and clinical malaria episodes will be determined. The frequency of COVID-19 will be compared between HIV-infected and HIV-uninfected women using a negative binomial regression. The proportion of women with adverse pregnancy outcomes will be compared by SARS-CoV-2 infection status using a modified binomial regression. These analyses will be done unadjusted and adjusted by baseline significant variables (age, gestational age, gravidity, RPR, anaemia and literacy, study intervention) and clinically relevant factors depending on the outcome for control of confounding factors. Incidences of hospital admissions in the neonate will be also analyzed using negative binomial regression. Data analysis will be performed using Stata (Stata Corp).[21]

Patient and public involvement. Patients will not be directly involved in the design, conduct, reporting or dissemination plans of the study.

DISCUSSION

At the onset of the COVID-19 pandemic, the extent of the risks in pregnancy was uncertain. In this context, the MA-CoV study was conceived to address fundamental questions on the burden and effects of SARS-CoV-2 infection during pregnancy. MA-CoV is an international, prospective observational cohort study that plans to follow pregnant women living in study areas of Gabon and Mozambique, where malaria and HIV infections are endemic and the real burden of SARS-CoV-2 infection is still unknown. Participants will be followed at monthly ANC visits, until 6 weeks after end of pregnancy. Additionally, the presence of antibodies (IgG/IgM) against SARS-CoV-2 in blood samples will be determined. The clinical presentation of COVID-19 in pregnancy will also be characterized, and incidence of infection during pregnancy will be evaluated, as well as the risk factors of maternal and neonatal morbidity and mortality associated with SARS-CoV-2 infection and the risk of mother-to-child transmission of SARS-CoV-2. Recruitment is expected to finish in September 2022, while patient follow-up is expected to be completed in May 2023.

The effects of SARS-CoV-2 infection on maternal and neonatal health have been described mostly by studies performed in high income countries, and include increased risk of admission to intensive care, abortion, c-section, pre-term birth, foetal growth restriction, post-partum hemorrhage and maternal mortality. [4-6] A retrospective cohort study analyzing routine data that was performed in six SSA countries reported similar findings.[22] Moreover, the clinical presentation of COVID-19 among non-pregnant women has been well described in the literature, which will allow us to compare our study findings with reports from settings with similar epidemiological characteristics. In addition, interaction of COVID-19 with other global epidemics such as HIV is particular relevant in the African region, given that it has the highest world incidence of HIV infection, being women of reproductive age at higher risk.[23] Recent studies have shown that HIV infection is associated with a significant increased risk of contracting SARS-CoV-2. In addition, immunosuppressed HIV-infected individuals have been shown to have higher incidence of severe COVID-19 and death than non-HIV-infected

individuals. [10 11 24] Importantly, the study performed in six countries of SSA found that pregnant women with HIV had an increased risk of admission to intensive care.[22]

Pregnant women still face disproportionate inequalities in access to and quality health care. The most essential maternal and reproductive health interventions do not reach yet the poorest and most vulnerable women, girls and children in the developing world. This results in marked poor understanding of the particular characteristics and health outcomes for this vulnerable group in many settings. The MA-CoV study constitutes a unique opportunity to improve the understanding of the effects of COVID-19 in pregnancy in pregnant women, while it will also assess other potential mechanisms of SARS-CoV-2 transmission such as vertical transmission during pregnancy and through breastfeeding. Thus, this study has the potential to produce an immediate beneficial public health impact at both regional and global level.

Limitations

The COVID-19 pandemic scenario presents several barriers and challenges not only due to the disease itself but also due to the implementation of containment measures such as quarantine, social distancing and community containment. To avoid this situation, relevant stakeholders will be meaningfully engaged from the very beginning and all throughout the process. Moreover, local and national health authorities' recommendations are being issued on the basis of ensuring continued provision of antenatal care, HIV prevention, testing, and treatment services.

Southern Mozambique is an area with high rates of population movement between countries such as Eswatini and South Africa, where women represent a large part in some areas.[25] Thus, some participants may be lost to follow-up despite efforts to reduce bias. However, this will be considered during the data analysis. In case of high rates of participants lost to follow-up, we will conduct analyses between the baseline characteristics of retained and lost participants.

ETHICS AND DISSEMINATION

The study is conducted in accordance with the European Medicines Agency (EMA)/International Council for Harmonization (ICH) Guideline on Good Clinical Practice and in total agreement with the applicable inter-national, European Union (EU) and national law of all the participating countries.46 The study protocol (V.1.0, 18 August 2021) and the informed consent forms have been reviewed and approved by the institutional and national ethics committees of Gabon (077/2021/CNE/SG/P) and Mozambique (61/CBNS/22) and the Hospital Clinic of Barcelona (HCB/2021/0942, Spain). The study is registered on clinicaltrials.gov (NCT05303168). The findings of the study will be submitted for publication in a peer-reviewed journal within 12 months of study completion through an open access mechanism, or otherwise made available publicly in compliance with H2020 open access requirements. Primary project raw data will be published in the project website.[26] At no stage will data containing personal information of research participants be released. After concluding the study's data analysis, findings will be made available to all partners, key stake-holders and Ministries of Health. The project members will actively disseminate information to the scientific community through reports, presentations at scientific forums and publications in international open-access journals.

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Authors contributions: RG conceived and designed the study; RG and AF-R wrote the study protocol. TN, GM-N, JM, ME, MR, SS, FS, and CM gave inputs to protocol methodology. AF-R, RG, GM-N, TN, MV, AM, MM, LM-N and BM were responsible for study conduct, reporting and

acquisition of data. AF-R and RG wrote the draft manuscript, all authors reviewed the draft and read and approved the final manuscript.

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References

1. WHO Coronavirus (COVID-19) Dashboard. Available: https://covid19.who.int [Accessed 28 July 2022].
2. Knight M, Bunch K, Vousden N, et al. Characteristics and outcomes of pregnant women admitted to hospital with confirmed SARS-CoV-2 infection in UK: national population based cohort study. *BMJ* 2020;369:m2107.
3. Wastnedge EAN, Reynolds RM, van Boeckel SR, et al. Pregnancy and COVID-19. *Physiol Rev* 2021;101(1):303-18.
4. Regan AK, Arah OA, Fell DB, et al. SARS-CoV-2 Infection During Pregnancy and Associated Perinatal Health Outcomes: A National US Cohort Study. *J Infect Dis* 2022;225(5):759-67.
5. Allotey J, Stallings E, Bonet M, et al. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis. *BMJ* 2020;370:m3320.
6. Villar J, Ariff S, Gunier RB, et al. Maternal and Neonatal Morbidity and Mortality Among Pregnant Women With and Without COVID-19 Infection: The INTERCOVID Multinational Cohort Study. *JAMA Pediatr* 2021;175(8):817-26.
7. Schwartz DA, Dhaliwal A. Coronavirus Diseases in Pregnant Women, the Placenta, Fetus, and Neonate. *Adv Exp Med Biol* 2021;1318:223-41.
8. Schwartz DA. An Analysis of 38 Pregnant Women with COVID-19, Their Newborn Infants, and Maternal-Fetal Transmission of SARS-CoV-2: Maternal Coronavirus Infections and Pregnancy Outcomes. *Arch Pathol Lab Med* 2020.
9. Ordi J, Bardaji A, Castillo P, et al. Grading schemes for placental malaria. *J Infect Dis* 2011;203(11):1694-5; author reply 95-6.
10. Ssentongo P, Heilbrunn ES, Ssentongo AE, et al. Epidemiology and outcomes of COVID-19 in HIV-infected individuals: a systematic review and meta-analysis. *Sci Rep* 2021;11(1):6283.
11. Hoffmann C, Casado JL, Harter G, et al. Immune deficiency is a risk factor for severe COVID-19 in people living with HIV. *HIV Med* 2021;22(5):372-78.
12. Gupta H, Macete E, Bulo H, et al. Drug-Resistant Polymorphisms and Copy Numbers in Plasmodium falciparum, Mozambique, 2015. *Emerging infectious diseases* 2018;24(1):40-48.
13. Gonzalez R, Nhampossa T, Mombo-Ngoma G, et al. Evaluation of the safety and efficacy of dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in HIV-infected pregnant women: protocol of a multicentre, two-arm, randomised, placebo-controlled, superiority clinical trial (MAMAH project). *BMJ Open* 2021;11(11):e053197.
14. Global Technical Strategy for Malaria 2016-2030. *World Health Organization* 2015. Available: https://www.who.int/docs/default-source/documents/global-technical-strategy-for-malaria-2016-2030.pdf?sfvrsn=c82afcc_0 [Accessed 20 July 2022].

15. Gonzalez R, Munguambe K, Aponte J, et al. High HIV prevalence in a southern semi-rural area of Mozambique: a community-based survey. *HIV Med* 2012;13(10):581-8. doi: 10.1111/j.1468-1293.2012.01018.x [published Online First: 20120415]
16. Manego RZ, Mombo-Ngoma G, Witte M, et al. Demography, maternal health and the epidemiology of malaria and other major infectious diseases in the rural department Tsamba-Magotsi, Ngounie Province, in central African Gabon. *BMC Public Health* 2017;17(1):130. doi: 10.1186/s12889-017-4045-x [published Online First: 20170128]
17. Elecsys® Anti-SARS-CoV-2. Roche. Available: https://diagnostics.roche.com/gb/en/products/params/elecsys-anti-sars-cov-2.html [Accessed 28 February 2023].
18. Elecsys® Anti-SARS-CoV-2 S. Roche. Available: https://diagnostics.roche.com/global/en/products/params/elecsys-anti-sars-cov-2-s.html [Accessed 28 February 2023].
19. Baud D, Greub G, Favre G, et al. Second-Trimester Miscarriage in a Pregnant Woman With SARS-CoV-2 Infection. *JAMA* 2020;323(21):2198-200. doi: 10.1001/jama.2020.7233
20. Pollan M, Perez-Gomez B, Pastor-Barriuso R, et al. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. *Lancet* 2020;396(10250):535-44.
21. StataCorp. Stata Statistical Software: Release 17. : College Station, TX: StataCorp LLC., 2021.
22. Nachega JB, Sam-Agudu NA, Machekano RN, et al. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection and Pregnancy in Sub-Saharan Africa: A 6-Country Retrospective Cohort Analysis. *Clin Infect Dis* 2022
23. HIV/AIDS | WHO | Regional Office for Africa. Available: https://www.afro.who.int/health-topics/hivaids [Accessed 15 May 2022].
24. Western Cape Department of Health in collaboration with the National Institute for Communicable Diseases SA. Risk Factors for Coronavirus Disease 2019 (COVID-19) Death in a Population Cohort Study from the Western Cape Province, South Africa. *Clin Infect Dis* 2021;73(7):e2005-e15.
25. IOM. World Migration Report. Geneva, 2022.
26. MA-CoV. Available: https://www.mamahproject.net/ma-cov-the-project/ [Accessed 03 June 2022].

Prevalence and impact of SARS-CoV-2 infection on maternal and infant health in African populations: protocol of a multi-centre prospective cohort study (MA-CoV project)

Supplementary material

File 1: Informed Consent of study

"Prevalence and impact of SARS-CoV-2 infection on maternal and infant health in African populations (MA-CoV)"

Introduction

The burden of COVID-19 is still unknown since access to diagnostic tests has been limited and therefore reserved for patients with severe disease and/or high-risk groups. In the African region, the number of reported cases is spreading and it is likely that vulnerable populations such as pregnant women and their foetuses will be directly and/or indirectly affected in the context of fragile health systems. It is important to understand the possible effects of COVID-19 on the health of pregnant and infants living in these regions to develop specific prevention measures.

Purpose and procedures of this study

The information coming from this MA-CoV study will help to understand the effects of the pandemic virus in African pregnant women. If you agree to be in the MA-CoV study, you will have a test done at the ***first antenatal care visit and in follow up visits*** in case you have symptoms or signs suggestive of COVID-19.

About the COVID-19 test

The test is a procedure called nasopharyngeal (NP) swab. The NP swab involves placing a swab (like a very long Q-tip) in your nose to collect cells and secretions. The swab will go into your nasal cavity, above the roof of your mouth. In some cases, the swab may only go into the nostril. The swab will be sent to a laboratory for testing to see if you are infected with COVID-19. The results of the COVID-19 testing will be made available to you, together with sufficient information to understand what the results mean. In case you are found to be infected, you will receive treatment free of charge and information regarding isolation and transmission prevention measures to be put in place at your home.

What happens during the study?

If you agree to be in this study, your first visit will continue today, after you read, discuss, and sign or put thumbprint on this form. You will be asked to come back to the clinic monthly before delivery. In addition, you must agree to deliver your baby at the study facility rather than at home.

If you agree to be in this study:

- We will first ask you some questions about yourself and your health
- We will ask you to give information on where you live and how to keep in contact with you
- A study clinician will examine you and will check your pregnancy status
- You will also be asked to give a venous blood sample at the first visit for tests of your blood (malaria and COVID-19 virus antibodies)
- In case you will be unwell with malaria or other infection, you will have additional blood tests done and if needed you will be given medicine and asked to come back here as scheduled by study staff
- You and your baby will receive a unique identification number (ID) and identification study card, which you will be requested to present to the study staff at every visit
- At delivery you will be visited during in the labour ward and you and your new-born baby will be examined by the study personnel.
- In addition to venous blood being collected from you, also a sample of cord blood will be taken to analyse the presence of COVID-19 virus antibodies
- A piece of placenta will be examined at the study laboratory and also tested for COVID-19 virus
- You must agree to deliver at the health facility but in case you deliver at home, the study staff will visit you as soon as possible but not later than one week after delivery and will ask you questions about your delivery and about health of your infant. At this visit you and your infant will be examined by the study personnel. Blood sample will be taken from you for tests of malaria
- We will ask you to provide us with a small sample of breastmilk (3 ml, less than a teaspoon) within three days and one month after your infant's birth to investigate if the virus can be found in maternal milk.
- When your baby is born, your child will be followed up until he/she is 1 month old
- You will be asked to come back with your new-born to the study clinic around 1 month after delivery to exam your baby and see if your baby is growing well

Other COVID-19 analyses and samples

We will also analyse the presence of the virus (which is called SARS-CoV-2) in the blood and placental samples that will be collected from you at enrolment and at the end of pregnancy. In case you are found to be infected with the COVID-19 virus, your infant will also be tested with a NP swab at birth. Also, if she/he presents with symptoms or signs suggestive of COVID-19 during her/his first month of life, she/he will have a test done and will receive the indicated treatment.

Alternatives to joining the MA-CoV study

If you choose not to participate in this study you will receive standard ANC care as before.

Risks or discomforts *(mother and infant)*

You might feel slight discomfort when we take nasopharyngeal swabs or venous blood samples at enrolment and delivery. There will be no other risks.

Benefits to you and your infant

By participating in the study, you may get better diagnosis of COVID-19 and other diseases such as malaria because of increased number of tests done. You and your baby will be regularly seen by clinical staff and in case of any symptoms or abnormal test results you and your baby will be either treated here or referred to another clinic for medical care.

STATEMENT of CONSENT AND SIGNATURE

Participant approval:
The consent form has been explained to me and I agree to take part in the MA-CoV study. I understand that I am free to choose to be in this activity and that saying "No" will not affect the treatment I get in this clinic, now and in future.
NOTE: You are not giving up any of your legal rights by signing this informed consent document.

If you agree circle YES

Volunteer's Name (print)	Volunteer's Signature or Thumbprint (if cannot write)	Date

Volunteer's Legal Guardian or Representative (as per country policy) (print)	Legal Guardian's Signature	Date

Witness's Name (if participant illiterate) (print)	Witness's Signature	Date

I have explained the purpose of this study to the volunteer. To the best of my knowledge, she understands the purpose, procedures, risks and benefits of this study.

Investigator/Designee Name (print)	Investigator/Designee Signature	Date

NOTE: This consent form with original signatures must be retained on file by the principal investigator. A copy must be given to the volunteer. If the woman refuses to take her copy of the consent with her, she states so below and signs and dates her decline statement.

File 2: Study case report forms

ISGlobal Barcelona Institute for Global Health MA-CoV Maternal health and COVID-19	**ID** MACOC- |__|-|__|__|__|__| Site Code Subject Number **Participant's initials** |__|__| |__| 1. 2. Family name

Prevalence and impact of SARS-CoV-2 infection on maternal and infant health in African populations (MA-CoV)

Project Acronym	MA-CoV

eCRF book ID MA-CoV
Version: v.3.1
Date: 28th April 2022

BIRTH

Newborn visit

Ma-COV **Event: Birth - Newborn visit**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

	INCLUSION CRITERIA CHECK	
1	Mother's ID	MACOW|__|-|__|__|__|__| Site Code Subject Nº
2	Date of birth	|__|__| - |__|__|__| - |__|__|__|__| Day Month Year
3	Sex	Masculine ☐ Feminine ☐
	MEDICAL HISTORY AND PHYSICAL EXAMINATION AT BIRTH	
4	Weight (g)	|__|__|__|__|__|
5	Length (cm)	|__|__|.|__|
6	Head circumference (cm)	|__|__|.|__|
7	Axillary temperature (ºC)	|__|__|.|__|
8	Congenital abnormalities?	Yes ☐ No ☐
	8.1. Face and head	Normal ☐ Abnormal ☐ Unknown ☐
	8.2. Limbs	Normal ☐ Abnormal ☐ Unknown ☐
	8.3. Chest	Normal ☐ Abnormal ☐ Unknown ☐
	8.4. Spine	Normal ☐ Abnormal ☐ Unknown ☐
	8.5. Abdomen	Normal ☐ Abnormal ☐ Unknown ☐
	8.6. Genitalia	Normal ☐ Abnormal ☐ Unknown ☐
	8.7. Other abnormalities	Yes ☐ No ☐
	8.7.1. If yes, describe	_____

If necessary, fill in the comments section

9	Does the child need admission to the hospital for any problem? *If the answer is yes please fill in an AE form*	Yes ☐ No ☐
10	10.1. Neuromuscular maturity	10.1.1 Posture score |__| 10.1.2 Square window |__| 10.1.3 Arm recoil |__| 10.1.4 Popliteal angle |__| 10.1.5 Scarf sign |__| 10.1.6 Heel to ear |__|
	Ballard test: 10.2. Physical maturity	10.2.1 Skin |__| 10.2.2 Lanugo |__| 10.2.3 Plantar surfasse |__| 10.2.4 Breast |__| 10.2.5 Eye-Ear |__| 10.2.6 Geniyals |__|
	THROAT SWAB **IF THE MOTHER'S PCR HAS BEEN POSITIVE AT PREGNANCY, PLEASE FILL IN THE FOLLOWING QUESTIONS**	
11	Was a throab swab for COVID-19 collected from the newborn?	Yes ☐ No ☐
12	IF yes, indicate the SARS-CoV-2 PCR result If positive, fill/update the Adverse Event Form	Positive ☐ Negative ☐
13	Was a rapid antigen test for COVID-19 performed?	Yes ☐ No ☐
14	IF yes, indicate the COVID-19 rapid antigen test result If Positive, fill/update the Adverse Event Form	Positive ☐ Negative ☐
	HIV PROPHYLAXIS **IF THE MOTHER TESTED POSITIVE FOR HIV**	
15	Has the newborn been given an ARV drug for HIV prophylaxis? *If yes, please fill out the Medication Form*	Yes ☐ No ☐

COMMENTS (OPTIONAL)

1 _____

2 _____

3 _____

4 _____

5 _____

6 _____

7 _____

8 _____

BIRTH

Newborn laboratory results

MA-CoV **Event: Newborn laboratory results**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

	SARS-CoV-2 PCR LAB RESULTS (baseline) – *if a nasopharyngeal swab was collected*	
1	Date of the sample	|__|__|- |__|__|__|-|__|__|__|__| Day Month Year
2	SARS-CoV-2 PCR test result *If positive, fill/update the Adverse Event Form*	Positive ☐ Negative ☐
3	Ct value	|__|__|
4	SARS-CoV-2 Viral load	|__|__|__|__|__|__| copies/mL

COMMENTS (OPTIONAL)

1 _____

2 _____

3 _____

4 _____

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POST-PARTUM VISIT

1 month after birth

Newborn questionnaire

MA-CoV **Event: Newborn laboratory results**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

	ATTENDANCE TO SCHEDULED VISIT	
1	Did the infant attend the scheduled visit to the health facility?	Yes ☐ No ☐
	1.1 *If the answer is no, please specify*	Migration ☐ Not found ☐ Absent ☐ Death ☐ Refused ☐ Other ☐ _____
	MEDICAL HISTORY AND PHYSICAL EXAMINATION	
2	Weight (g)	|__|__|__|__|__|
3	Length (cm)	|__|__|.|__|
4	MUAC (cm)	|__|__|.|__|
5	Head circumference (cm)	|__|__|.|__|
6	Axillary temperature (ºC)	|__|__|.|__|
7	Does the infant have a congenital abnormality not previously diagnosed?	Yes ☐ No ☐
8	Has the infant been admitted to the hospital since the last visit?	Yes ☐ No ☐
	COVID INFECTION SUSPICION **INQUIRY OF SIGNS AND SYMPTOMS DURING THE FIRST MONTH OF LIFE**	
9	Cough?	Yes ☐ No ☐
10	Fever? (Tª ≥ 37,5 ºC)	Yes ☐ No ☐
11	Shortness of breath?	Yes ☐ No ☐
12	Rhinorrhea?	Yes ☐ No ☐
13	Does the participant's legal guardian report fever during the last 24 hours?	Yes ☐ No ☐
	If Temp ≥ 37,5 ºC or the answer is YES for any of the questions, collect a throat swab for COVID-19	
14	Was a throat swab for COVID-19 collected? *If yes, please complete SARS-CoV-2 PCR lab results in the Laboratory Results Form*	Yes ☐ No ☐
15	Was a rapid antigen test for COVID-19 performed?	Yes ☐ No ☐
16	If yes, indicate the COVID-19 rapid antigen test result *If Positive, fill/update the Adverse Event Form*	Positive ☐ Negative ☐

	NUTRITION	
	Is the woman breastfeeding the infant?	Yes ☐ No ☐
17	17.1 If yes, please specify when breastfeeding started	Less than an hour after birth ☐ Between 1 and 12 hours after birth ☐ Between 12 and 24 hours after birth ☐ More than 24 hours after birth ☐
	During the first month of life, did the infant receive other foods or beverages apart from breast milk?	Yes ☐ No ☐
18	18.1 If yes, please specify which foods or beverages he/she received	*Water* ☐ *Juice* ☐ Other type of milk ☐ *Vegetables* ☐ *Fruit* ☐ *Sweets or sugar* ☐ *Traditional herbs* ☐ *Rice or cereals* ☐ *Other* ☐ Please specify: _____
	Yesterday, did the infant receive other foods or beverages apart from breast milk?	Yes ☐ No ☐
19	19.1 If yes, please specify which foods or beverages he/she received	*Water* ☐ *Juice* ☐ Other type of milk ☐ *Vegetables* ☐ *Fruit* ☐ *Sweets or sugar* ☐ *Traditional herbs* ☐ *Rice or cereals* ☐ *Other* ☐ Please specify: _____
	PSYCHOMOTOR DEVELOPMENT ASSESSMENT	
20	Was the psychomotor development assessed?	Yes ☐ No ☐
	Gross motor skills	
21	21.1 Does the infant move the 4 extremities symmetrically?	Yes ☐ No ☐
	21.2 Muscle tone	Normal ☐ Abnormal ☐
	Fine motor skills	
22	Does the infant follow objects?	Yes ☐ No ☐
	Language / audition	
23	Does the infant respond to sounds?	Yes ☐ No ☐
	Social skills	
24	Does the infant respond to smiles?	Yes ☐ No ☐

	COMMENTS (OPTIONAL)
1	_____
2	_____
3	_____
4	_____

POST-PARTUM

1 month after birth

Newborn laboratory results

MA-CoV NCT03671109 **Event: Laboratory results Newborn**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

	SARS-CoV-2 PCR LAB RESULTS (1 month after birth)	
1	Date of the sample	|__|__|- |__|__|__|-|__|__|__|__| Day Month Year
2	SARS-CoV-2 PCR test result *If positive, fill/update the Adverse Event Form*	Positive ☐ Negative ☐
3	Ct value	|__|__|
4	SARS-CoV-2 Viral load	|__|__|__|__|__|__| copies/mL
	HIV PCR LAB RESULTS – *if a HIV PCR was done*	
5	Was an HIV PCR done?	Yes ☐ No ☐
5.1	*If yes,* Date of the sample	|__|__|- |__|__|__|-|__|__|__|__|
5.2	HIV PCR test result *If positive, fill/update the Adverse Event Form*	Positive ☐ Negative ☐

COMMENTS (OPTIONAL)

1 _____

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STUDY COMPLETION FORM

STUDY COMPLETION FORM MA-CoV **Event: Study completion form Newborn**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

	STUDY COMPLETION	
1	Date of last contact?	|__|__| - |__|__|__| - |__|__|__|__| Day Month Year
2	Did the newborn complete the study? *2.1 If the answer is no, please provide all relevant information related to reason for premature discontinuation*	Yes ☐ No ☐ Death ☐ Serious health outcome ☐ Consent withdrawal ☐ Migration ☐ Lost to follow up ☐ Other ☐ Specify: _____
3	Date of participant's study completion	|__|__| - |__|__|__| - |__|__|__|__| Day Month Year
4	I have reviewed and found all data pertaining to this participant to be complete and accurate Printed Investigator's name _____	|__|__| - |__|__|__| - |__|__|__|__| Day Month Year

Please provide all relevant information related to reason for premature study discontinuation including contributory factors in the comments section

COMMENTS (OPTIONAL)

1 _____

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ADVERSE EVENTS FORM

MA-CoV	**ID** MACOC|__|-|__|__|__|__|__| Site Code Subject Nº	**Participant's initials** |__|__| |__| 1. 2. Family name	

Severity: **1** Mild (Grade 1): Awareness of sign or symptom easily tolerated, **2** Moderate (Grade 2): Discomfort enough to cause interference with usual activity, **3** Severe (Grade 3): Incapacitating with inability to work or perform usual activity, **4** Life-threatening (Grade 4): Patient at risk of death at the time of the event, or Event Results in death, or Requires hospitalization or prolongation of existing hospitalization, or Results in persistent or significant disability or incapacity or Consists of a congenital anomaly or birth defect

Outcome: 1-Completely recovered, 2-Not yet completely recovered, 3-Deterioration, 4-Permanent damage, 5-Death, 6-Ongoing, 7-Unknown

Action Taken: 1-No action taken, , 2-Concomitant medication given, 3-Non-drug therapy given, 4-Hospitalization/Hospitalization prolonged

ADVERSE EVENT #			
1	A. Name / Description |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|	B. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	C. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Month Year
2	A. Severity ☐ 1 ☐ 2 ☐ 3 ☐ 4	B. Outcome ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7	C. Action taken ☐ 1 ☐ 2 ☐ 3 ☐ 4
3	Is this AE Serious? ☐NO ☐YES		
ADVERSE EVENT #			
1	A. Name / Description |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|	B. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	C. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Month Year
2	A. Severity ☐ 1 ☐ 2 ☐ 3 ☐ 4	B. Outcome ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7	C. Action taken ☐ 1 ☐ 2 ☐ 3 ☐ 4
3	Is this AE Serious? ☐NO ☐YES		
ADVERSE EVENT #			
1	A. Name / Description |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|	B. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	C. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Month Year
2	A. Severity ☐ 1 ☐ 2 ☐ 3 ☐ 4	B. Outcome ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7	C. Action taken ☐ 1 ☐ 2 ☐ 3 ☐ 4
3	Is this AE Serious? ☐NO ☐YES		

MEDICATION FORMS

MA-CoV Medication forms	ID MACOC|__|-|__|__|__|__| Site Code Subject Nº	Participant's initials |__|__| |__| 1. 2. Family name	

	Medication #1			
1	A. Medication/ Non-drug therapy |__|__|__|__|__|__|__|__|__|	B. Dosage |__|__|__|__|__|__|	C. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	D. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Day Month Year
2	A. Reason to take it _____	B. Is an AE the cause of taking it? ☐YES (fill an AE form) ☐NO, the cause started prior to recruitment		

	Medication #2			
1	A. Medication/ Non-drug therapy |__|__|__|__|__|__|__|__|__|	B. Dosage |__|__|__|__|__|__|	C. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	D. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Day Month Year
2	A. Reason to take it _____	B. Is an AE the cause of taking it? ☐YES (fill an AE form) ☐NO, the cause started prior to recruitment		

	Medication #3			
1	Medication/ Non-drug therapy |__|__|__|__|__|__|__|__|__|	B. Dosage |__|__|__|__|__|__|	C. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	D. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Day Month Year
2	A. Reason to take it _____	B. Is an AE the cause of taking it? ☐YES (fill an AE form) ☐NO, the cause started prior to recruitment		

	Medication #4			
1	A. Medication/ Non-drug therapy |__|__|__|__|__|__|__|__|__|	B. Dosage |__|__|__|__|__|__|	C. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	D. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Day Month Year
2	A. Reason to take it _____	B. Is an AE the cause of taking it? ☐YES (fill an AE form) ☐NO, the cause started prior to recruitment		

ISGlobal Barcelona Institute for Global Health

MA-COV Maternal health and COVID-19

ID MACOC- |__|-|__|__|__|__|
Site Code Subject Number

Participant's initials |__|__| |__|
1. 2. Family name

Prevalence and impact of SARS-CoV-2 infection on maternal and infant health in African populations (MA-CoV)

Project Acronym	MA-CoV

eCRF book ID MA-CoV
Version: v.3.1
Date: 28th April 2022

BIRTH

Newborn visit

Ma-COV **Event: Birth - Newborn visit**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

	INCLUSION CRITERIA CHECK	
1	Mother's ID	MACOW|__|-|__|__|__|__| Site Code Subject Nº
2	Date of birth	|__|__| - |__|__|__| - |__|__|__|__| Day Month Year
3	Sex	Masculine ☐ Feminine ☐
	MEDICAL HISTORY AND PHYSICAL EXAMINATION AT BIRTH	
4	Weight (g)	|__|__|__|__|__|
5	Length (cm)	|__|__|.|__|
6	Head circumference (cm)	|__|__|.|__|
7	Axillary temperature (ºC)	|__|__|.|__|
8	Congenital abnormalities?	Yes ☐ No ☐
	8.1. Face and head	Normal ☐ Abnormal ☐ Unknown ☐
	8.2. Limbs	Normal ☐ Abnormal ☐ Unknown ☐
	8.3. Chest	Normal ☐ Abnormal ☐ Unknown ☐
	8.4. Spine	Normal ☐ Abnormal ☐ Unknown ☐
	8.5. Abdomen	Normal ☐ Abnormal ☐ Unknown ☐
	8.6. Genitalia	Normal ☐ Abnormal ☐ Unknown ☐
	8.7. Other abnormalities	Yes ☐ No ☐
	8.7.1. If yes, describe	_____

If necessary, fill in the comments section

9	Does the child need admission to the hospital for any problem? *If the answer is yes please fill in an AE form*	Yes ☐ No ☐
10	10.1. Neuromuscular maturity	10.1.1 Posture score |__| 10.1.2 Square window |__| 10.1.3 Arm recoil |__| 10.1.4 Popliteal angle |__| 10.1.5 Scarf sign |__| 10.1.6 Heel to ear |__|
	Ballard test: 10.2. Physical maturity	10.2.1 Skin |__| 10.2.2 Lanugo |__| 10.2.3 Plantar surfasse |__| 10.2.4 Breast |__| 10.2.5 Eye-Ear |__| 10.2.6 Geniyals |__|
	THROAT SWAB **IF THE MOTHER'S PCR HAS BEEN POSITIVE AT PREGNANCY, PLEASE FILL IN THE FOLLOWING QUESTIONS**	
11	Was a throab swab for COVID-19 collected from the newborn?	Yes ☐ No ☐
12	IF yes, indicate the SARS-CoV-2 PCR result If positive, fill/update the Adverse Event Form	Positive ☐ Negative ☐
13	Was a rapid antigen test for COVID-19 performed?	Yes ☐ No ☐
14	IF yes, indicate the COVID-19 rapid antigen test result If Positive, fill/update the Adverse Event Form	Positive ☐ Negative ☐
	HIV PROPHYLAXIS **IF THE MOTHER TESTED POSITIVE FOR HIV**	
15	Has the newborn been given an ARV drug for HIV prophylaxis? *If yes, please fill out the Medication Form*	Yes ☐ No ☐

COMMENTS (OPTIONAL)

1 _____

2 _____

3 _____

4 _____

5 _____

6 _____

7 _____

8 _____

BIRTH

Newborn laboratory results

MA-CoV **Event: Newborn laboratory results**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

SARS-CoV-2 PCR LAB RESULTS (baseline) – *if a nasopharyngeal swab was collected*		
1	Date of the sample	|__|__|- |__|__|__|-|__|__|__|__| Day Month Year
2	SARS-CoV-2 PCR test result *If positive, fill/update the Adverse Event Form*	Positive ☐ Negative ☐
3	Ct value	|__|__|
4	SARS-CoV-2 Viral load	|__|__|__|__|__|__| copies/mL

COMMENTS (OPTIONAL)

1 _____

2 _____

3 _____

4 _____

5 _____

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7 _____

8 _____

POST-PARTUM VISIT

1 month after birth

Newborn questionnaire

MA-CoV **Event: Newborn laboratory results**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

	ATTENDANCE TO SCHEDULED VISIT	
1	Did the infant attend the scheduled visit to the health facility?	Yes ☐ No ☐
	1.1 *If the answer is no, please specify*	Migration ☐ Not found ☐ Absent ☐ Death ☐ Refused ☐ Other ☐ _____
	MEDICAL HISTORY AND PHYSICAL EXAMINATION	
2	Weight (g)	|__|__|__|__|__|
3	Length (cm)	|__|__|.|__|
4	MUAC (cm)	|__|__|.|__|
5	Head circumference (cm)	|__|__|.|__|
6	Axillary temperature (ºC)	|__|__|.|__|
7	Does the infant have a congenital abnormality not previously diagnosed?	Yes ☐ No ☐
8	Has the infant been admitted to the hospital since the last visit?	Yes ☐ No ☐
	COVID INFECTION SUSPICION **INQUIRY OF SIGNS AND SYMPTOMS DURING THE FIRST MONTH OF LIFE**	
9	Cough?	Yes ☐ No ☐
10	Fever? ($T^a \geq 37,5$ ºC)	Yes ☐ No ☐
11	Shortness of breath?	Yes ☐ No ☐
12	Rhinorrhea?	Yes ☐ No ☐
13	Does the participant's legal guardian report fever during the last 24 hours?	Yes ☐ No ☐
	If Temp ≥ 37,5 ºC or the answer is YES for any of the questions, collect a throat swab for COVID-19	
14	Was a throat swab for COVID-19 collected? *If yes, please complete SARS-CoV-2 PCR lab results in the Laboratory Results Form*	Yes ☐ No ☐
15	Was a rapid antigen test for COVID-19 performed?	Yes ☐ No ☐
16	If yes, indicate the COVID-19 rapid antigen test result *If Positive, fill/update the Adverse Event Form*	Positive ☐ Negative ☐

	NUTRITION	
	Is the woman breastfeeding the infant?	Yes ☐ No ☐
17	17.1 If yes, please specify when breastfeeding started	Less than an hour after birth ☐ Between 1 and 12 hours after birth ☐ Between 12 and 24 hours after birth ☐ More than 24 hours after birth ☐
	During the first month of life, did the infant receive other foods or beverages apart from breast milk?	Yes ☐ No ☐
18	18.1 If yes, please specify which foods or beverages he/she received	*Water* ☐ *Juice* ☐ Other type of milk ☐ *Vegetables* ☐ *Fruit* ☐ *Sweets or sugar* ☐ *Traditional herbs* ☐ *Rice or cereals* ☐ *Other* ☐ Please specify: _____
	Yesterday, did the infant receive other foods or beverages apart from breast milk?	Yes ☐ No ☐
19	19.1 If yes, please specify which foods or beverages he/she received	*Water* ☐ *Juice* ☐ Other type of milk ☐ *Vegetables* ☐ *Fruit* ☐ *Sweets or sugar* ☐ *Traditional herbs* ☐ *Rice or cereals* ☐ *Other* ☐ Please specify: _____
	PSYCHOMOTOR DEVELOPMENT ASSESSMENT	
20	Was the psychomotor development assessed?	Yes ☐ No ☐
	Gross motor skills	
21	21.1 Does the infant move the 4 extremities symmetrically?	Yes ☐ No ☐
	21.2 Muscle tone	Normal ☐ Abnormal ☐
	Fine motor skills	
22	Does the infant follow objects?	Yes ☐ No ☐
	Language / audition	
23	Does the infant respond to sounds?	Yes ☐ No ☐
	Social skills	
24	Does the infant respond to smiles?	Yes ☐ No ☐

COMMENTS (OPTIONAL)

1 _____

2 _____

3 _____

4 _____

POST-PARTUM

1 month after birth

Newborn laboratory results

MA-CoV NCT03671109 **Event: Laboratory results Newborn**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

	SARS-CoV-2 PCR LAB RESULTS (1 month after birth)	
1	Date of the sample	|__|__|- |__|__|__|-|__|__|__|__| Day Month Year
2	SARS-CoV-2 PCR test result *If positive, fill/update the Adverse Event Form*	Positive ☐ Negative ☐
3	Ct value	|__|__|
4	SARS-CoV-2 Viral load	|__|__|__|__|__|__| copies/mL
	HIV PCR LAB RESULTS – *if a HIV PCR was done*	
5	Was an HIV PCR done?	Yes ☐ No ☐
5.1	*If yes,* Date of the sample	|__|__|- |__|__|__|-|__|__|__|__|
5.2	HIV PCR test result *If positive, fill/update the Adverse Event Form*	Positive ☐ Negative ☐

COMMENTS (OPTIONAL)

1 _____

2 _____

3 _____

4 _____

5 _____

6 _____

7 _____

8 _____

STUDY COMPLETION FORM

STUDY COMPLETION FORM MA-CoV **Event: Study completion form Newborn**	**ID** MACOC|__|-|__|__|__|__| Site Code Subject Nº **Participant's initials** |__|__| |__| 1. 2. Family name **Date of the visit** |__|__|- |__|__|__|-|__|__|__|__| Day Month Year	

	STUDY COMPLETION	
1	Date of last contact?	|__|__| - |__|__|__| - |__|__|__|__| Day Month Year
2	Did the newborn complete the study?	Yes ☐ No ☐
	2.1 If the answer is no, please provide all relevant information related to reason for premature discontinuation	Death ☐ Serious health outcome ☐ Consent withdrawal ☐ Migration ☐ Lost to follow up ☐ Other ☐ Specify: _____
3	Date of participant's study completion	|__|__| - |__|__|__| - |__|__|__|__| Day Month Year
4	I have reviewed and found all data pertaining to this participant to be complete and accurate	
	Printed Investigator's name _____	|__|__| - |__|__|__| - |__|__|__|__| Day Month Year

Please provide all relevant information related to reason for premature study discontinuation including contributory factors in the comments section

COMMENTS (OPTIONAL)

1 _____

2 _____

3 _____

4 _____

5 _____

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7 _____

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9 _____

10 _____

ADVERSE EVENTS FORM

MA-CoV	ID MACOC|__|-|__|__|__|__| Site Code Subject Nº	Participant's initials |__|__| |__| 1. 2. Family name	

Severity: **1** Mild (Grade 1): Awareness of sign or symptom easily tolerated, **2** Moderate (Grade 2): Discomfort enough to cause interference with usual activity, **3** Severe (Grade 3): Incapacitating with inability to work or perform usual activity, **4** Life-threatening (Grade 4): Patient at risk of death at the time of the event, or Event Results in death, or Requires hospitalization or prolongation of existing hospitalization, or Results in persistent or significant disability or incapacity or Consists of a congenital anomaly or birth defect

Outcome: 1-Completely recovered, 2-Not yet completely recovered, 3-Deterioration, 4-Permanent damage, 5-Death, 6-Ongoing, 7-Unknown

Action Taken: 1-No action taken, , 2-Concomitant medication given, 3-Non-drug therapy given, 4-Hospitalization/Hospitalization prolonged

ADVERSE EVENT #			
1	A. Name / Description |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|	B. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	C. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Month Year
2	A. Severity ☐ 1 ☐ 2 ☐ 3 ☐ 4	B. Outcome ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7	C. Action taken ☐ 1 ☐ 2 ☐ 3 ☐ 4
3	Is this AE Serious? ☐NO ☐YES		

ADVERSE EVENT #			
1	A. Name / Description |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|	B. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	C. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Month Year
2	A. Severity ☐ 1 ☐ 2 ☐ 3 ☐ 4	B. Outcome ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7	C. Action taken ☐ 1 ☐ 2 ☐ 3 ☐ 4
3	Is this AE Serious? ☐NO ☐YES		

ADVERSE EVENT #			
1	A. Name / Description |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|	B. Start date |__|__| |__|__|__||__|__|__|__| Day Month Year	C. End date |__|__| |__|__|__||__|__|__|__| ☐ Ongoing Month Year
2	A. Severity ☐ 1 ☐ 2 ☐ 3 ☐ 4	B. Outcome ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7	C. Action taken ☐ 1 ☐ 2 ☐ 3 ☐ 4
3	Is this AE Serious? ☐NO ☐YES		

MEDICATION FORMS

MA-CoV Medication forms	ID MACOC|__|-|__|__|__|__| Site Code Subject Nº	Participant's initials |__|__| |__| 1. 2. Family name	

Medication #1

	A. Medication/ Non-drug therapy	B. Dosage	C. Start date	D. End date
1	|__|__|__|__|__|__|__|__|__|	|__|__|__|__|__|__|	|__|__| |__|__|__||__|__|__|__| Day Month Year	|__|__| |__|__|__||__|__|__|__| ☐ Ongoing Day Month Year
2	A. Reason to take it _____	B. Is an AE the cause of taking it? ☐YES (fill an AE form) ☐NO, the cause started prior to recruitment		

Medication #2

	A. Medication/ Non-drug therapy	B. Dosage	C. Start date	D. End date
1	|__|__|__|__|__|__|__|__|__|	|__|__|__|__|__|__|	|__|__| |__|__|__||__|__|__|__| Day Month Year	|__|__| |__|__|__||__|__|__|__| ☐ Ongoing Day Month Year
2	A. Reason to take it _____	B. Is an AE the cause of taking it? ☐YES (fill an AE form) ☐NO, the cause started prior to recruitment		

Medication #3

	Medication/ Non-drug therapy	B. Dosage	C. Start date	D. End date
1	|__|__|__|__|__|__|__|__|__|	|__|__|__|__|__|__|	|__|__| |__|__|__||__|__|__|__| Day Month Year	|__|__| |__|__|__||__|__|__|__| ☐ Ongoing Day Month Year
2	A. Reason to take it _____	B. Is an AE the cause of taking it? ☐YES (fill an AE form) ☐NO, the cause started prior to recruitment		

Medication #4

	A. Medication/ Non-drug therapy	B. Dosage	C. Start date	D. End date
1	|__|__|__|__|__|__|__|__|__|	|__|__|__|__|__|__|	|__|__| |__|__|__||__|__|__|__| Day Month Year	|__|__| |__|__|__||__|__|__|__| ☐ Ongoing Day Month Year
2	A. Reason to take it _____	B. Is an AE the cause of taking it? ☐YES (fill an AE form) ☐NO, the cause started prior to recruitment		