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Characterising transmission of SARS-CoV-2 in a peri-urban population in Mozambique using population-based (sero) surveillance

AfriCoVER

Protocol
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Sponsor:	Institute of Tropical Medicine Nationalestraat 155 B-2000 Antwerpen - Belgium
Coordinating Investigator:	Dr Marc-Alain Widdowson

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Sponsor study team

Institute of Tropical Medicine, Belgium

Name	Function	Role
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Harry Van Loen	Data Manager	Set-up and follow-up of study databases, advice on Good Data Management practices, data review, drafting of Data Management Plan
Yven Van Herrewege	Head of the CTU	Development of GCP compliant research protocol, ICF and other essential documents, clinical project management, follow-up and coordination of study management performed by the Clinical Trials Unit
Joachim Mariën	Investigator	conceptual development and validation of serology, revisions of the protocol and questionnaires, virology and modelling expertise, day-to-day study development (virology; serology), data analysis (serology; PCR)
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Name	Function	Role
Dr. Ivalda Macicame	Principal Investigator	Revisions of the protocol and questionnaires, leading the project ensuring that the implementation of the study follows the plan described in the protocol and to high standards of scientific rigor, ensure that data analysis conforms to high standards of scientific rigor and ensure the write-up of the results for appropriate dissemination.
Dr. Edna Viegas	Investigator	Contribute to the design and implementation of the project following high standards of scientific rigor. Participate in data analysis and report writing and dissemination.
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Ferao Mandlate	Study Coordinator	Implement the study in an ethical manner, including protecting the rights, welfare and privacy of human subjects who are participants in study. Participate in data analysis and report writing and dissemination.
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Dr. Nalia Ismael	Laboratory Coordinator	Implement the study according to the Good Clinical and Laboratory l Practices, ensure sample collection, analyses and storage.

External partners

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Name	Function	Role
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Name	Function	Role
Dr. Mirjam Kretzschmar	Investigator	conceptual development of transmission models, data analysis (transmission parameters; epidemic modelling including population structure and social mixing patterns)

STATEMENT OF COMPLIANCE

By signing this protocol, the Investigator(s) acknowledge(s) and agree(s):

This protocol contains the necessary information for conducting this clinical study. The Principal Investigator will conduct this study as detailed herein and will make every reasonable effort to complete the study within the time designated. The Principal Investigator commits to carry out the study in compliance with the protocol, amendments, applicable procedures and other study-related documents provided by the Sponsor, and in compliance with the Declaration of Helsinki, Good Clinical [Laboratory] Practice (GC[L]P), the EU General Data Protection Regulation 2016/679 (GDPR), the ESF/ALLEA Code of Conduct for Research Integrity, and applicable regulatory requirements.

The protocol and all relevant study information, which is provided by the Sponsor, will be made available to the physicians, nurses and other personnel who participate in conducting this study. The Investigator will use this material for their training so that they are fully informed regarding the drugs and the conduct of the study.

The Sponsor of this study – the Institute of Tropical Medicine in Antwerp, Belgium (ITM) – can at any time have access to the source documents from which Case Report Form information may have been generated and will be permitted to perform trial-related monitoring and audits. All study material will be maintained according to regulatory requirements and until the Sponsor advises that retention is no longer necessary.

COORDINATING INVESTIGATOR ITM AND DIRECTOR OF THE ITM:			
	Title, Name: Dr. Marc-Alain Widdowson	Date:	
	Signed: 		
PRINCIPAL INVESTIGATOR INS:			
	Title, Name: Dr. Ivalda Macicame	Date:	
	Signed:		

Signing this document, I commit to carry out the trial in accordance with the protocol, Good Clinical Practice and applicable ethical and regulatory requirements. I also acknowledge the paragraph relevant to study confidentiality and authorize the Institute of Tropical Medicine, Antwerp, Belgium to record my data on a computerized system containing all the data pertinent to the study.

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Synopsis

design	Prospective cohort study (population-based surveillance and serial sero-survey)
study site & population	Polana Caniço, peri-urban Maputo, Mozambique: a health- and demographic surveillance site (HDSS) covering a population of approximately 15,393, in the catchment area of the Polana Caniço General Hospital (PCGH). All individuals residing in the 2108 HDSS households for at least 3 months (infants, children, adults, elderly) are included in bi-weekly surveillance rounds, regardless of age. An age-stratified random sample of that population (800 per age stratum) will be selected for the repeated sero- and social mixing survey. No exclusion criteria apply for participants able and willing to provide consent for participation in the study.
duration	December 2020-December 2021 (12 months)
objectives and endpoints	Primary objectives ● Estimate the attack rate, serial interval, and reproduction number of SARS-CoV-2 infection in peri-urban Maputo. ● Estimate the proportion of infections that are asymptomatic, and tease out the role these play in transmission. ● Estimate the incidence rate of SARS-CoV-2 infection, COVID-19 disease (symptomatic infection), rate of severe COVID-19 disease, hospitalization for medical care and mortality, over a 12 month period in peri-urban Maputo ● Identify risk factors for SARS-CoV-2 infection, for asymptomatic or clinical presentation, and for COVID-19-related death, which could include HIV status, nutritional status, blood group, underlying co-morbidities, household factors, pre-existing levels of anti-alphacoronavirus antibodies (CoV 229E, NL63) or anti-betacoronavirus antibodies (CoV OC43 and HKU1), and availability and adoption of measures aimed to reduce transmission. ● Create social contact matrices that can be used to parametrize dynamic transmission models. ● Estimate the household secondary infection rate Secondary objectives ● Measure antibody titers over time and analyze the correlation between anti-coronavirus (endemic coronavirus or SARS-CoV-2) antibody titers and reinfection ● Assess the individual uptake of measures aimed to reduce transmission (quarantine, physical distancing, hygiene, future vaccination) and assess barriers to uptake (absence soap/water, income loss, ability to self-isolate, availability of masks, distrust, lack of understanding of the communication) ● Derive and validate a clinical prediction rule for SARS-CoV-2 infection. ● Geographical mapping of clusters, spread and identification of transmission hotspots ● Sequence selected strains of SARS-CoV-2

	● Understand non-SARS-CoV-2 etiologies of respiratory disease in the study population ● Identify factors associated with household SARS-CoV-2 infection and with household infection resulting in Covid19 disease, including viral load and symptoms of household index case, household and individual characteristics and behaviour to prevent infection. ● Estimate total (of vaccinees) and overall (including indirect protection of non-vaccinated) vaccine effectiveness among the study population, once one or more vaccines are introduced
screening, recruitment & randomization	All household members will be screened for symptoms during biweekly household visits. Individuals reporting any respiratory sign, or loss of smell or taste, with or without fever (either presenting at the COVID-19 care facility or identified through the biweekly household visits) will provide specimens that will undergo PCR testing. Symptoms, clinical outcome, vaccination status against COVID-19, and potential exposure will be recorded. Three age-stratified subsets of the population (0-17 years; 18-50 years; >50 years) will be recruited for a sero-survey at 0, 3, 6 and 12 months and a social mixing survey, repeated 4 times during the outbreak. Household contacts of household cases testing positive for SARS-CoV-2 will be screened for virus and antibodies and for illness 1 day after confirmation of the household index case and 28 days after index case symptom onset
follow-up	The clinical outcome of individuals with a confirmed SARS-CoV-2 infection (mild cases at home; hospitalised cases in the hospital or after discharge) will be recorded during the weekly visits and at 28 and 56 days post symptom onset.
analytical methods	* Description of transmission dynamics: rolling incidence rate of COVID-19 disease throughout one year, adjusting for the sensitivity of SARS-CoV-2 RT-PCR on nasal swabs by delay between symptom onset and testing; SARS-CoV-2 infection incidence rate, SARS-CoV-2 annual attack rate, secondary infection rate, and infection fatality risk by age group, by adjusting for the proportion asymptomatics. * Risk factor analysis for SARS-CoV-2 infection and COVID-19 disease, and for severity (hospitalisation), overall and following household transmission in particular. * Model basic and effective reproductive numbers (R0 and Re), generation time distribution, ratio of transmissibility between symptomatic and asymptomatic cases, duration of the infectious period, household secondary infection rate. * Explore the correlation between R0 and Re, and the uptake of measures to reduce exposure/ transmission. * Case-cohort, case- control or similar analysis to estimate vaccine effectiveness against confirmed COVID-19 in the population-based surveillance and cohort analysis to estimate vaccine effectiveness against SARS-CoV-2 seroconversion (among sero-survey participants)
safety	The study does not involve an investigational product or intervention

statistical methods	Descriptive (probabilistic) and inferential statistics Negative binomial regression for risk factor analysis Development of a compartmental mathematical model to describe the spread of SARS-CoV-2 on the population level

1. Introduction

1.1 Background

In sub-Saharan Africa, official Covid-19 case numbers are an underestimate because of limited surveillance and testing capacity, whereby testing, when it happens, is not systematic and focuses on high probability suspects, either severe, imported cases or their contacts. This provides a very limited and biased assessment of transmission and burden in the population and hence public health impact, also hampering implementation of targeted interventions (Ahmed A, 2020). Furthermore, co-morbidities (such as HIV or TB) and malnutrition are more common in sub-Saharan Africa and are likely to affect disease dynamics and outcomes. Despite concerns of a possible pending catastrophic impact of Covid-19 on Africa, there is also still a pervasive hope that perhaps younger populations in warmer, humid tropical Africa, living mainly outdoors, might be less affected, which has been postulated as an explanation as to why months after the initial reported clusters of cases, so far still no or limited widespread transmission has been reported in many sub-Saharan African countries (Casanova et al 2020; Notari A 2020).

Nevertheless, experience from other enveloped respiratory RNA viruses such as influenza virus shows that respiratory transmission can occur throughout the year in tropical Africa with occasional usually erratic peaks (Gessner et al 2011). In Maputo, Mozambique, slightly south of the Tropic of Capricorn, influenza transmission is more seasonal once again, though in most years, two peaks can be observed (Nguenha et al 2018). Despite unclear seasonality in much of Africa, age-stratified estimates of mortality rate of the 2009 pandemic influenza were as high if not higher as elsewhere globally (Dawood et al 2011). Similarly, archival studies have estimated a huge societal impact of the 1918 influenza pandemic on the health of African populations (Andayi F 2019). Moreover, epidemiologic studies on endemic human coronaviruses in Kenya have shown substantial year-round circulation (Munywoki et al 2018). This suggests that SARS-CoV-2 could have a high impact in Africa, although SARS-CoV-2 is different in key ways to influenza virus and to other coronaviruses. Data from higher income country contexts is poorly applicable to African settings. Some lower resourced southern hemisphere settings have already seen large increases in cases in early April, such as Ecuador, but in the Southern temperate region, we only started to observe high intensity transmission from the end of May onwards.

Several demographic, cultural and environmental factors may affect the transmission of SARS-CoV-2 in communities and within households in Africa. These include population density, population age structure, poor-quality housing (including exposure to indoor smoke), climate, different intensity and pattern of contact (e.g. between the ages), different distribution of co-morbidities, different exposures to vaccines and infections, but also the ability to adhere to interventions aimed at interrupting transmission, such as physical distancing (including quarantine and isolation), use of masks and of water/soap for hand-washing. In addition, host factors may affect transmission. For instance, HIV-infected persons with low CD4 counts may shed longer as is the case with influenza (von Mollendorf et al 2018), and there may be differences in ACE2 receptors (the binding receptor for SARS-CoV-2) densities across populations (Cao et al 2020) or other genetic factors (Ellinghaus et al preprint 2020). Host factors and co-morbidities more prevalent or specific to Africa could also affect to what extent an individual presents with asymptomatic or clinical illness, which may also affect transmissibility and infectiousness (Cohen et al 2015).

Knowledge about transmission parameters will allow for the design of targeted, specific control measures to interrupt spread of SARS-CoV-2 through behaviour modification, social distancing and use of personal protective measures. In addition, intensity of transmission and prevalence of risk groups for infection and disease translates to population level burden of disease and the incidence rate of that

burden in communities. Understanding the number of events of relative severity, size of risk groups for severe disease and the current and projected rate of change of disease is critical to plan for current and future capacity for health care.

The transmission potential of SARS-CoV-2 is determined in any particular context by the basic reproduction number (R0) when there is no protective immunity in the population. R0 will vary with time and context, such as population density, number of contacts and environmental conditions, and is dependent on the period of infectiousness, viral load, and the serial interval. Early data estimated the R0 for SARS-CoV-2 between 2.0 and 3.4 (Ferretti 2020; Li 2020), higher than that for seasonal influenza (Biggerstaff et al 2014). This is likely in part because of key divergent features from influenza such as pre and asymptomatic SARS-CoV-2 transmission (Ferretti 2020; Kimball 2020; He 2020), near absence of severe disease in young children (Wu et al 2020), and a longer estimated serial interval of 3.96 days (Du Z Emerging Infect Dis 2020). The SARS-CoV-2 average R0 is also higher than estimated for previous novel viruses, such as influenza H1N1 in the 1918 (R0=1.8) and 2009 (R0=1.6) pandemics (Biggerstaff et al 2014) and SARS-CoV-1 (Ro=1-5) (Liu et al 2020).

To date, evidence suggests that in temperate climates between 20 and 50% of infections could be asymptomatic (Mizumoto et al 2020; Bi et al 2020; Kimball et al 2020). Pre-symptomatic shedding is common, with comparable viral load in asymptomatic, pre-symptomatic and clinical cases (Kimball et al 2020). This complicates timely targeted application of interventions since people may not know they are infected and will not behave as if they were. The frequency of asymptomatic infection in sub-Saharan African settings is as of yet unclear, and also may vary by setting. Understanding the proportion of asymptomatic infections and their contribution to the reproduction number is important.

Immune factors may affect community transmission. Cross reactivity with existing endemic coronaviruses, (CoVs 229E, NL63, HKU1,OC43), two of which are related beta-coronaviruses (HKU1,OC43), could result in protection from infection and/or disease. Many models assume a person can no longer be infected once recovered (Fig 1). However, immunity to coronaviruses is unclear. Antibody titer to SARS-CoV-1 infection waned over a period of several months in recovered patients (Liu et al 2006), but unclear if this means waning protection. Additionally, endemic human coronaviruses existing neutralising antibodies may not sufficiently protect against reinfection (Callow et al 1985), while some reports of reinfection with SARS-CoV-2 have been reported, though none fully documented.

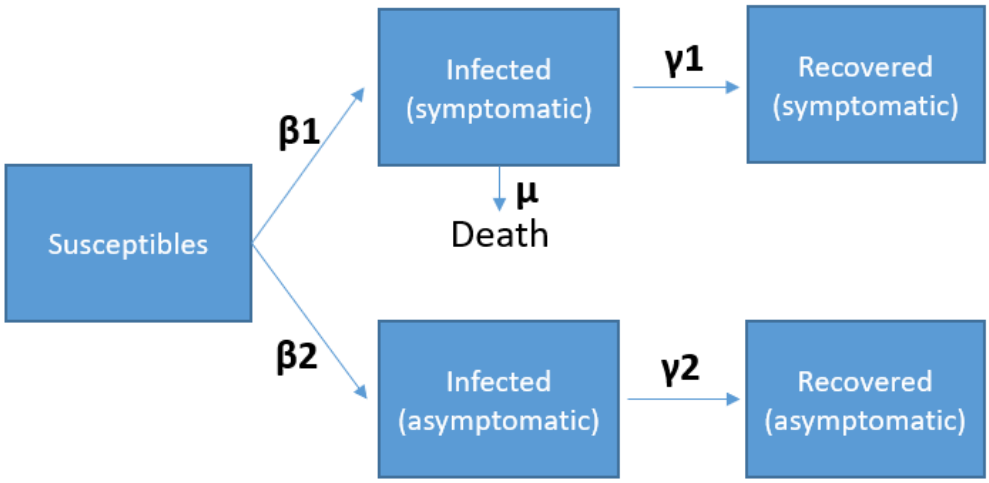

Fig 1. Susceptible-Infected-Recovered Model

In the first few weeks of 2021, vaccines under WHO's Emergency Use Listing have begun to be used in several countries and plans exist to roll out one or more vaccine types in Mozambique. Vaccine effectiveness studies are key to better understand how these vaccines perform in different settings, especially since the emergence of SARS-CoV-2 variants circulating in Mozambique against which several current vaccines may not protect.

1.2 Rationale

It is recognized that SARS-CoV-2 will transmit differently in African settings, and that risk factors for infection and mild or severe disease may be different to higher income settings. Understanding transmission and identifying at-risk populations is urgent for African countries, who need to target scarce resources as effectively as possible to interrupt transmission and protect vulnerable risk groups.

Box. The epidemiological situation in Mozambique on 29/09/2020.
The SARS-Cov-2 outbreak is still accelerating in Mozambique, as indicated by both the trend in the number of reported cases and the positivity rate. 7983 SARS-CoV-2 infected cases were reported, including 295 imported cases, and 58 deaths. Since the start of the outbreak, 134,011 suspected cases have been tested. In September 2020, consistently more than half of new cases were reported in Maputo City. In Maputo City a total of 3,427 cases and 38 deaths have been reported. The average number of new daily cases reported went from 47 in the first week of September, to 97 in the week up to 26/09. A sero-prevalence survey undertaken in the last two weeks of August 2020, found an overall seroprevalence of 3.8% in Mauto city, with only slight variation between age groups (range 3.0% in 35-59 year olds to 4.5% in ≥60 year olds). Nevertheless, the latter age group accounts for the majority of hospitalisations. Of all cases reported in Mozambique, 56% were asymptomatic, 37% have a mild symptomatology, 6% moderate symptomatology and 1% a severe symptomatology. 42% of the infected individuals are female.

We aim to determine robust transmission parameters of SARS-CoV-2 to understand infectiousness and epidemic spread. We will investigate risk factors for infection and severe disease, assess impact of vaccines / behavioral interventions on transmissibility and infectiousness, and explore the role of certain age groups, societal activities, pre- and asymptomatic infections in transmission in a peri-urban area of Maputo, Mozambique. By building on an existing health and demographic surveillance system (HDSS) it will be highly feasible to expand the existing population-based surveillance with biweekly home visits, viral RNA and serological testing. For serological testing, a newly validated SARS-CoV-2 antibody assay will be added to the capacity at INS. This will provide high quality, timely, unbiased epidemiological data and indicators on the natural history and key SARS-CoV-2 transmission parameters, which can be rapidly collected and analysed, informing the country's response, as well as that of other African countries.

Since the number of cases started to accelerate in September 2020, we aim to have a first analysis point at 3 months after the implementation of the project in the HDSS, to estimate the progression of the pandemic in the community and help plan for effective and feasible control measures.

2. Study Objectives

Primary objectives

- Estimate the attack rate, infection fatality, serial interval, and reproduction number of SARS-CoV-2 infection in peri-urban Maputo.
- Estimate the proportion of infections that are asymptomatic, and tease out the role these play in transmission.

- Estimate the incidence rate of SARS-CoV-2 infection, COVID-19 disease (symptomatic infection), rate of severe COVID-19 disease, hospitalization for medical care and mortality, over a 12 month period in peri-urban Maputo.
- Identify risk factors for SARS-CoV-2 infection and for severe COVID-19 disease, which could include reported HIV status, nutritional status, blood group, underlying co-morbidities, household factors, pre-existing levels of anti-alphacoronavirus antibodies (CoV 229E, NL63) or anti-betacoronavirus antibodies (CoV OC43 and HKU1), and availability and adoption of measures aimed to reduced transmission.
- Create social contact matrices that can be used to parametrize dynamic transmission models.
- Estimate the household secondary infection rate

Secondary objectives

- Measure antibody titers over time and analyze the correlation between anti-coronavirus (endemic coronavirus or SARS-CoV-2) antibody titers and reinfection
- Assess the individual uptake of measures aimed to reduce transmission and assess barriers to uptake (absence soap/water, income loss, ability to self-isolate, availability of masks, distrust, lack of understanding of the communication)
- Derive and validate a clinical prediction rule for SARS-CoV2 infection
- Geographical mapping of clusters, spread and identification of transmission hotspots.
- Sequence selected strains of SARS-CoV-2
- Understand non-SARS-CoV-2 etiologies of respiratory disease in the study population.
- Identify factors associated with household SARS-CoV-2 infection and with household infection resulting in Covid19 disease, including viral load and symptoms of household index case, household and individual characteristics and behaviour to prevent infection.
- Estimate total brand-specific vaccine effectiveness (among vaccinees) against PCR confirmed SARS-CoV-2 clinical infection.
- Estimate total brand-specific vaccine effectiveness (among vaccinees) against SARS-CoV-2 seroconversion
- Estimate overall and brand-specific vaccine effectiveness (total among vaccinees and indirect among non-vaccinated) against PCR confirmed SARS-CoV-2

Table 1. Study activities and outcome measures. Primary outcome measures in **bold**.

Planned activities	Sampling strategy and sample size	Outcome measures
Active syndromic population surveillance (biweekly) with PCR testing of symptomatic individuals – 12 months	15393 individuals (2108 households) Possible cases reporting any respiratory sign or loss of smell or taste, with or without fever, selected for PCR testing. Initially, before widespread community transmission, pooled testing of possible cases; when >50 confirmed cases per week, switch to PCR testing of 1 in 10 possible cases (systematic selection - every 10th possible case)	● **COVID-19 disease incidence rate (symptomatic infections per person-month)** ● COVID-19 related hospitalisation rate ● Case (disease) fatality risk (%) ● Risk factors for severe outcome/hospitalization ● Basic reproduction number ● Effective reproduction number ● Serial interval ● Proportion uptake of measures to prevent infection; barrier uptake ● Effectiveness of interventions

		● COVID-19 incidence density maps ● COVID-19 likelihood ratios of clinical signs ● Brand- specific vaccine e ffectiveness against confirmed COVID-19 (disease)
Age-stratified sero-survey at 0, 3, 6, 12 months	2400 individuals/dried blood spot samples per round (800 per age group <18yo, 18-50 yo, >50yo) An age stratified sample of individuals will be selected (simple random sample in each age group)	● **SARS-CoV-2 infection annual attack rate (%)** ● Infection fatality risk ● Proportion asymptomatic infections, by age group ● Risk factors for infection ● Association between antibody titers and reinfection ● Brand-specific total vaccine effectiveness against SARS-CoV-2 seroconversion ● Overall vaccine effectiveness against SARS-CoV-2 seroconversion
Repeated social mixing survey	the same 2400 individuals selected for the sero-survey (800 per age group <18yo, 18-50 yo, >50yo)	● correlation between number of contacts, intensity of contacts and attack rate ● correlation between uptake of control measures and attack rate ● social contact matrices to be used to parametrize dynamic transmission models
Household contact investigation at 1 day after confirmation of the household index case and 28 days after index case symptom onset	First 100 households with ≥1 confirmed case; median household size 7.3 => 100*6= 600 household contacts	● Household secondary infection rate ● Risk factors for household infection ● Risk factors for household infection resulting in Covid19 disease ● Total vaccine effectiveness against COVID-19

3. Study Design

Prospective cohort study (observational). We will use the established HDSS to set up population-based COVID-19 surveillance in a household cohort. From any individual in this population reporting any respiratory sign (symptom or lowered peripheral oxygen saturation) or loss of smell or taste, with or without fever, we will collect a respiratory specimen and test these for SARS-CoV-2 virus by PCR, during 12 months. These cases will be identified through bi-weekly household visits, through an alert system involving community leaders and HDSS interviewers, or when presenting with symptoms at healthcare centres or at the referral hospital. Identified healthcare facilities will then alert the study team if a possible case from the HDSS population is consulted or admitted, if the patient agrees. Demographic, epidemiological and clinical data (comorbidities, medication, obesity, HIV, TB, smoking) of possible cases and of the source population will be recorded at baseline; recent illness, potential risk exposure of each household member (including duration and type) to SARS-CoV-2 positive individuals, uptake of measures to reduce exposure/transmission, and barriers to the uptake of such measures, will be

recorded/updated during every household visit. COVID-19 vaccination status will be recorded from possible cases and compared to that of a subset of the population, in a case-cohort design to determine vaccine effectiveness against symptomatic COVID-19 .

An age-stratified serial sero-survey will be conducted at baseline in a subset of the population in the population-based COVID-19 surveillance, and at 3, 6 and 12 months, after confirmation of community transmission. Recording vaccination status of all sero-survey participants, we will determine vaccine effectiveness against SARS-CoV-2 seroconversion in a cohort design.

Household transmission will be specifically investigated, by investigating contact history, vaccination status, and the presence of SARS-CoV-2 virus and serology among household members twice during the month after the symptom onset of a household index case.

4. Participants, Population & Selection

4.1 Setting

Polana Caniço, peri-urban Maputo, Mozambique: a health- and demographic surveillance system (HDSS) covering a population of 15,393, in the catchment area of the Polana Caniço General Hospital (PCGH), one out of two COVID-19 referral facilities in Maputo.

The population in peri-urban Polana Caniço is relatively representative of the increasingly urban population in Southern Africa, having young and mobile inhabitants. Moreover, the population suffers increasingly from non-communicable diseases, and prevalence of HIV and active tuberculosis is presumed to be important. Research gaps on COVID-19 transmission dynamics include whether the reproduction number could be different in such different (eg. younger) population. Also, the potential association between (uncontrolled) HIV infection or (active) tuberculosis and COVID-19 infection and severe disease, needs to be elucidated.

The Polana Caniço-HDSS has been established since 2017. In total, as of time of writing, 2108 households (defined as residents of the same building, sharing a cooking area) have been enrolled in the HDSS. All members residing in the household for at least 3 months (infants, children, adults, elderly) are enrolled in the HDSS. Surveillance rounds are currently carried out six-monthly. Demographic and health indicators collected in each surveillance round include but are not limited to: fertility/birth rate, mortality/cause of death, migration, disease cases, trauma and violence, socioeconomic status, water and sanitation facilities, risk factors for the acquisition of chronic diseases, women's health and family planning, immunization, risk behaviours and cultural practices related to the transmission of STI (including HIV and syphilis). Testing for HIV and syphilis is planned to be added as part of the surveillance rounds, from October 2020 onwards. Data on births, deaths, internal and external migration, pregnancies and their outcomes, and any other relevant events from a demographic perspective, are continuously reported by community key informants and confirmed through home visits.

4.2 Selection and recruitment

All households in the HDSS (2108 during the last round in 2019) consenting to participate in the study will be **visited biweekly** by a trained HDSS interviewer, following the same procedures as in routine HDSS rounds, specified in the HDSS protocol (Instituto Nacional de Saude, 2016). We assume the same number of households will consent to participate as in other HDSS rounds. Based on previous experience with the HDSS, we expect the HDSS interviewer to visit 6 households per working day on average.

Household heads, or an adult delegated by the household head, will be asked about COVID-19 consistent symptoms among any consented household member, using a structured questionnaire. If a household member is identified by the household head as reporting any respiratory sign (symptom or lowered peripheral oxygen saturation) or loss of smell or taste, with or without fever, in the last week, i.e. a possible COVID-19 case, this household member will be asked to consent for participating, to respond to a structured questionnaire and to collect a nasal swab sample for SARS-CoV-2 testing. Specimens will be tested for SARS-CoV-2, using RT-PCR (at INS in Marracuene). Possible COVID-19 cases in the HDSS households will be detected through four entry points:

- Biweekly HH visits
- Phone call by patient (when symptoms occur)
- Phone call by study team (as currently done in HDSS)
- HDSS household members presenting as COVID-19 suspected case in healthcare facility

If meeting the COVID-19 admission criteria as per national guidance, the possible COVID-19 case will be referred to the Polana Caniço General Hospital (PCGH), the closest healthcare facility and COVID-19 patient management centre.

An **age-stratified random sample** of that population will be selected for the **repeated sero- and social mixing survey**. By age stratum (0-17 years; 18-50 years; >50 years) we randomly select 800 individuals (simple random sample per age stratum; 2400 in total) from the Polana Caniço HDSS population (regardless of infection or disease status or history), from whom during home visits dried blood spots will repeatedly be collected at 0, 3, 6 and 12 months, vaccination status against COVID-19 will be recorded, and who will be asked to respond to a social mixing questionnaire. A HDSS sampling frame of all individual participants in the HDSS with socio-demographic data and unique identifiers, is available, and will be used to select a simple random sample of individuals by age group.

When a member of a household with no prior widespread transmission (see exclusion criteria) tests SARS-CoV-2 positive, we will revisit the household twice for a household transmission investigation: as soon as possible after the result of the household index case (baseline), and once again one month after symptom onset of the household index case. An initial contact report form will be filled in as soon as possible, ideally within 24 hours, after laboratory confirmation of the primary case in the household. Respiratory and dried blood spot samples will be collected of everyone considered resident (as defined by the HDSS) in the household and who has been in contact with the SARS-CoV-2 positive individual, regardless of age and presence or absence of symptoms. Respiratory samples will be analysed by RT-PCR for SARS-CoV-2 to assess presence of virus and viral load. The dried blood spots for serology on the Luminex assay. Data will be collected on household exposures of contacts and vaccine receipt. Symptomatic household contacts will be tested whenever symptoms appear, as part of the population-based surveillance. This is planned for upto 100 household index cases.

4.3 Inclusion and exclusion criteria

In order to be eligible, study participants **must meet the following criteria**:

- Any individual enrolled in the Polana Caniço-HDSS: all members residing in the household for at least 3 months (infants, children, adults, elderly), regardless of age, underlying conditions, medical history, infection or disease status or history;
- Able and willing to provide written informed consent: by the household head for the surveillance; by each selected participant for the sero-survey and household investigations.
- For household investigations, over 50% of the eligible household members and index case should have provided consent for anyone in the household to enrol.

Excluded from the household investigations: members of households in which more than 3 household members (including the index case) are known to have tested positive for SARS-CoV-2 in the past 7 days.

4.4 Sample size

See table 1, above, and justification for the sample size calculation in section 7.4 Sample size and power.

4.5 Withdrawal and termination of the study

Reasons for Withdrawal

Participants may be withdrawn from the study if the participant or legally acceptable representative withdraws the consent. For participants who consented to participate, but who moved out of the study area during the study period, the months when residing in the study area will still be considered.

Handling of Withdrawals

When the investigator has no news of the participant, he/she must make every effort to contact him/her, to establish the reason for the discontinuation. If all these attempts to contact the participant fail, the investigator can then declare the participant "lost to follow-up". The investigator should document all these attempts in the corresponding medical file.

Termination of Study

If the study is prematurely terminated or suspended, the Sponsor will promptly inform the investigators/institutions, and the regulatory authority(ies) of the termination or suspension and the reason(s) for the termination or suspension. The EC's will also be informed promptly and provided the reason(s) for the termination or suspension by the Sponsor or by the investigator/institution, as specified by the applicable regulatory requirement(s).

5. Study Procedures

5.1 Study/visit schedule

Table 2. Schedule of assessments for population-based surveillance. One row corresponds to two weeks.

week	month	Informed consent (1)	Sero-survey	Household visit (2)	Unscheduled visit for swab collection	Case Follow-up outcome (3)	Social mixing & uptake (4)
1	Oct20	x	x	x		x	
3				x	x	x	
5	nov/20			x	x	x	x
7				x	x	x	
9				x	x	x	

11	dec/20			x	x	x	
13			x	x	x	x	
15	jan/21			x	x	x	
17				x	x	x	x
19	feb/21			x	x	x	
21				x	x	x	
23	mar21			x	x	x	
25			x	x	x	x	x
27	apr/21			x	x	x	
29				x	x	x	
31	may21			x	x	x	
33				x	x	x	
35				x	x	x	
37	jun/21			x	x	x	
39				x	x	x	
41	jul/21			x	x	x	
43				x	x	x	
45	aug/21			x	x	x	
47				x	x	x	
49	sep/21			x	x	x	x
51			x	x	x	x	
53						x	
55						x	
TOTAL forms filled*		2800 for household surveillance,2400 for sero-survey,8000 for swab collection	2400*4= 9600	2800*5= 70000	8000	8000	2400*4= 9600
TOTAL samples*			2400*4= 9600		8000		

Type of sample		DBS for serology, collected at home**		nasal swab for PCR, collected at home**		

DBS = dried blood spot
1 Informed consent for household surveillance (by the household head) and sero-survey (by the selected individual or their caretaker) during baseline visit

2 During baseline visit adapted questionnaire to update population characteristics

3 Only of COVID-19 confirmed cases identified by the surveillance, at 28 and 56 days after symptom onset

4 We plan the social mixing survey rounds each time one month after the sero-survey, to spread the workload. The same participants from the sero-survey are asked to complete the questionnaire
* estimated, based on the available information at the start of the study; we anticipate 8000 respiratory episodes (possible cases), based on previous data from the HDSS, adjusted for increased frequency of reporting (previous data was recorded during three-monthly rounds)
** both serology testing of DBS and PCR testing of nasal swabs will be carried out at INS Marracuene. DBS samples are collected during pre planned visits, PCR samples when a possible case is identified. Nasal swabs undergo PCR testing the day following sample collection and results are communicated back to the individual (or his/her caretaker) that same day.

All HDSS households will be visited by a trained HDSS interviewer to present the study and ask for informed consent (i) by the household head for the population-based surveillance and (ii) by the individuals selected for the age-stratified serial sero-survey. During this same visit, if the household head consented, the demographics data (baseline household visit form) will be verified and updated. A study household card with unique IDs of each household member will be provided (code containing the housing block, household id and sequential household member number). Household members will be sensitized on symptoms related to COVID-19, are asked to keep track of possible symptoms they may develop, and inform the study team by phone if a household member has a respiratory symptom, loss of smell or taste, so that upon assessment he/she can be asked to be swabbed for virological testing. In case a household member presents with symptoms which classify him/her as a possible case, a separate individual informed consent procedure for nasal swabbing will have to be performed prior to sampling. This ICF covers sampling for the first episode and any subsequent episode during the study period when nasal sampling would be indicated. Also during the baseline household visit, if full informed consent was provided (both for surveillance by the household head and for the sero- and social mixing survey by the individual household member), individual household members selected for the sero-survey will be asked for a baseline dried blood spot sample on filter paper, by administering a few blood drops through a finger prick.

Every two weeks a trained interviewer from the HDSS team will do calls to the head of the household or his representative, where during the call he will fill out a questionnaire (Household follow-up visit form) recording any respiratory symptoms, fever or other indicative sign of COVID-19 (at the time of the phone call, or in the last two weeks, if these symptoms go away), vaccination against COVID-19, and medical appointments that any family member has had.

Meanwhile (if applicable), demographic changes or changes in the chronic medical conditions of family members will be recorded. If a family member reports a respiratory sign or loss of smell or taste, with or without fever (possible case), a trained interviewer will do a home visit to this household.

In case that the head of the household or his representative is not available by phone (number disconnected or unreachable or without contact details), an interviewer will perform a visit for this

household. In addition to calls, home visits to households will be performed every three months to collect data in person.

Any adolescent or adult able to self-administer a nasal swab will be asked to do so, at the participants home. For children or adults not capable of self-swabbing, the trained interviewer will put on personal protective equipment and collect a nasal swab. Preferentially, if confidentiality allows, the swab will be taken outside. Alternatively in a room without other household members. A 'Possible case report form', based on the WHO case report form, will be completed, recording symptoms, recent risk exposure, and potential contact with COVID-19 cases.

If in between household visits, a household member can inform the study team by phone when he/she develops any sign that could be indicative of COVID-19, so that a trained interviewer will be dispatched to assess whether the signs qualify for a possible case, complete the 'Possible case report form', and collect a nasal swab (again by self-swabbing if possible) at the possible cases' home.

If during household visits or through phone call, the interviewer is notified that a participating household member has been admitted to a healthcare facility or presented to the COVID-19 clinical management centre at Polana Caniço General Hospital, the participant will be actively approached, and if applicable, a 'Possible case report form' filled. If SARS-CoV-2 PCR testing has been done already, results will be requested and entered in a 'Possible case lab request and result form'. If no SARS-CoV-2 PCR testing has been done yet, a nasal swab will be collected if indicated, following the same procedure as above.

Household investigations will be held , whenever a household index case) is identified, excluding households in which more than 3 household members (including the index case) are known to have tested positive for SARS-CoV-2 in the past 7 days. During household transmission investigation visits, all eligible household members will be proposed to self-administer a nasal swab if adolescent or adult, or a nasal swab will be collected by the interviewer if the participant is a child or unable to administer a nasal swab. A Dried blood spot sample will be collected. The household head will be requested to keep track of any clinical sign or symptom of household members during the month following the baseline visit, in a diary, and if so, notify the study team. For symptomatic household participants, study procedures of a possible case report, as detailed above, will be followed. 28 days after symptom onset of the household index case, again a nasal swab and DBS sample will be collected for each household member.

Table 3. Schedule of assessments and samples collected for household transmission investigations.

	Day0 Onset of symptoms of index case	Day X Diagnosis of index case	Day X+1 Sample collection from contacts	Day 28 28 days after onset of index
Index case		nasal swab	DBS	nasal swab + DBS
Household contacts			nasal swab + DBS	nasal swab + DBS

Collected swabs will be stored in viral transport media, kept on conditioned ice packs (0-8°C), then shipped twice a day to a fridge (2-8°C) in the CISPOC office, from where they will be dispatched for PCR-testing at INS in a cooled container (2-8°C) once a day.

Results of PCR testing will be reported once daily from the lab to the national COVID-19 surveillance (also coordinated by INS) and to the study team, who will within two hours inform the possible case on his/her (confirmed or negative) SARS-CoV-2 PCR result by phone and, if an infection is confirmed, provide instructions how to protect household members and when to seek specialized COVID-19 care at the Polana Caniço hospital (according to national guidance).

Confirmed cases will be followed up by phone 28 days and 56 days after symptom onset, to record clinical outcome, using the 'Confirmed case follow-up form'. If not possible to connect by phone, this will be filled in during the subsequent household visit, or through follow-up with the COVID-19 clinical management centre at Polana Caniço General Hospital (if the patient died or is still hospitalised).

The repeated sero-survey, collecting dried blood sports through a finger prick, will be added to the biweekly household visits at 3, 6 and 12 months after COVID-19 local transmission is confirmed. During four other biweekly household visits, the participants selected for the sero-survey will also be interviewed on contact and mobility patterns during the previous day, and uptake of COVID-19 control measures, using the 'Social mixing questionnaire'. Its timing will depend on the stage of the outbreak:

1. shortly after the baseline interview (second visit),
2. when local transmission is well established and the number of new cases is increasing,
3. a month after an initial peak, when the number of new cases is decreasing,
4. during the final study visit.

Table 4 . Forms completed during each study activity

Planned activities	**Sample size**	**Research form**
Baseline household visit	15393 individuals (2108 households)	· Informed consent form for population-based COVID-19 surveillance (with household head) · Informed consent form for serial sero-survey (with selected individual) · Baseline household visit form
Active syndromic population surveillance	15393 individuals (2108 households) visited every 2 weeks	· Household follow-up visit form
Swabbing & PCR testing of symptomatic individuals	possible cases from the surveillance population; initially pooled testing of possible cases; when >50 confirmed cases per week, switch to PCR testing of 1 in 10 suspected cases	· Possible case report form · Possible case lab request and result form
Follow-up of PCR confirmed cases at day 28 and day 56 past symptom onset	PCR confirmed cases from the surveillance population	· Confirmed case follow-up form
Age-stratified sero-survey at 0, 3, 6, 12 months	2400 individuals/serum samples per round (800 per age group <18yo, 18-50 yo, >50yo NOTE: this is budget dependent, pending cost of	· Sero-survey form · Sero-survey lab request and result form

	assay, might be decreased to 1600, spread over two age strata)	
Social mixing survey	the same 2400 individuals selected for the sero-survey; during different HH visits than sero-survey visits	· Social mixing questionnaire
Household transmission investigation	100 household primary cases and their household members (who have been in contact with the index case	· baseline household transmission form

Table 5 . Samples collected during each surveillance and sero-survey study visit

Study visit Tests to carry out	Baseline(M0)	Biweekly visit	M3	Biweekly visit	M6	Biweekly visit	M9	Biweekly visit	M12	possible case visit	case follow-up visit (D28)	case follow-up visi (D56)
NASAL SWAB												
RT-PCR to detect SARS-Cov-2 viral RNA	*	*	*	*	*	*	*	*	*	X		
DRIED BLOOD SPOT (DBS)												
Luminex (anti-SARS-CoV-2serology)	X		X		X				X			
Approx. volume of collected blood	450ul		450ul		450ul				450ul			

* A nasal swab will only be collected if an individual is identified as a possible COVID19 case (respiratory symptoms, loss of smell or taste, with or without fever). All tests will be carried out at the INS-Marracuene lab in Mapûto, Mozambique.

5.2 Obtaining informed consent

The participation of each household in the health demographic surveillance system currently in place in Mozambique is strictly voluntary and is documented by signature of the head of the household or his/her designee (person aged no less than 18 years) on behalf of all household members on an informed consent form for the registration of each household in the system. During the baseline surveillance household visit, study specific written informed consent for the COVID-19 surveillance will be requested to the household head, as also requested for other sporadic studies integrated within the HDSS (Instituto Nacional de Saude, 2016). During the same visit, a separate informed consent will be requested of individuals (or their caretakers) selected for the sero-survey. For minors we will ask informed consent from parents or guardians, for minors of 12 years or older we will also have a written assent form. In case of illiteracy, a fingerprint from the illiterate study participant and a signature from a witness (person aged no less than 18 years that is not part of the HDSS team) will be obtained in all ICFs.

At the first study visit that a household member reports symptoms that classify this person as a possible case we will ask to sign a separate informed consent form to collect nasal swabs. This informed consent will cover for this first nasal swab sampling, but also for future indicated nasal swabs up to the end of the last AfriCoVER COVID-19 household surveillance visit. For minors we will ask informed consent from parents or guardians, for minors of 12 years or older we will also have a written assent form. In case of illiteracy, a fingerprint from the illiterate study participant and a signature from a witness (person aged no less than 18 years that is not part of the HDSS team) will be obtained on all ICFs.

For household transmission investigations, a separate individual informed consent will be requested to each participating household member. Only households participating and consenting to the population-based surveillance, and where at least 50% of household members enrol can be selected for household transmission investigations.

A 10% refusal to participate will be anticipated when selecting the simple random sample of individuals for the sero-survey.

After signing the informed consent the household and/or the individual participant is free to withdraw from participation in the study at any time.

The informed consent procedure will describe the (legitimate) purpose of the study, the procedures to be followed, the risks and benefits of participation, etc. If a participant (or parent or guardian) is unable to read or write, a signature from a witness to the informed consent discussion will be obtained. Participants (or parents or guardians) will be informed that participation in the study is completely voluntary and that the participant can withdraw from the study at any time without any negative consequences. All Informed consent forms (ICFs) will be available in Portugese and the informed consent procedure will also take place in Portuguese.

5.3 Specific procedures and activities

Collection of a nasal swab for SARS-CoV-2 PCR testing

Nasal swabs will be collected through self-swabbing under supervision of trained study staff (at >1.5m distance), or by the trained study staff member (in case of children or preference by participant).

1. Write the unique HDSS household member identifier on the tube, adding: 'N_Afri_cover_day' and stick a ID label from the household participant card on the tube.
2. (if the study staff is collecting the sample) Put on personal protective equipment

3. Insert the swab gently in a vertical position, 1-1.5 cm into the nostril, until there is gentle resistance
4. Rotate against the inside of the nostril for 10-15 seconds, to absorb secretions, while applying pressure with a finger to the outside of the nostril
5. Repeat the procedure in the other nostril with the same swab
6. Submerge the nasal swab in 1-3ml sterile saline or 1.5ml of UTM medium (Capricorn Scientific) in an Eppendorf tube
7. Keep in a cooled container with conditioned ice packs (0-8°C)
8. Ship within 4 hours to the CISPOC office, where samples are refrigerated at 4°C before shipping

The tubes with swabs will be stored at 2-8°C frigobox during transport to the INS lab and kept at this temperature if they can be tested within 72 hours. If a delay in testing or shipping is expected, specimens will be stored at -20°C or below. The samples can be stored at room temperature (15–30 °C) for up to 8 hours and refrigerated (2– 8 °C) up to seven days until testing is performed (information from GeneXpert Instrument Systems).

Dried blood spot for serology on Luminex

Anti-SARS-COV-2 antibodies will be checked on DBS based on a fingerpick. DBS are easy-to-collect in the field and experience has shown that antibodies can be detected with similar sensitivity as serum (especially with Luminex which only requires a low input amount: 1µl per sample). Furthermore, the INS staff and residents of the HDSS are more experienced in taking blood samples with a finger prick procedure, as they used this approach during previous studies (e.g. on HIV and malaria).

Blood spots will be collected through finger pricks by trained study staff.

1. Write the unique HDSS household member code on each filter paper, write a number 0 to 4, representing the sequence of surveys on the filter paper and the zipped bag, and stick a ID label from the household participant card on the zipped bag
2. Put on personal protective equipment, including gloves
3. Use a finger prick device to finger- or heel-prick blood, until a large droplet of blood forms on the skin. Apply six blood spots (75µl each) on filter paper (903 whatman protein saver cards)
4. Let the DBS sample air dry on a clean and dry surface, for 4 hours at ambient temperature
5. Store in a separate zipped bag with desiccant to reduce humidity damage (one per participant)
6. Store in a cool-box and ship at the end of the day to the central laboratory of INS or the CISPOC office, where they will be kept in a -20°C freezer.

5.4. Laboratory procedures and analyses

5.4.1 Data Collection

All participants will have unique coded identifiers for the construction of a pseudonymized database for third parties. These identifiers will be made separately for the identification of the nasal swab and Dried Blood Spot (DBS) collections of each survey. All samples will be transported to the central laboratory of the INS for analysis with the Luminex platform (serology) or 7500 (RT-PCR). Pre-analytical, analytical and post-analytical work related to the study samples will be carried out in compliance with Good Clinical Laboratory Practice (GCLP). All samples will be stored at INS and, under this protocol, only be used for the detection of coronavirus viral RNA or antibodies. Both nasal swabs and DBS will be stored long-term (5 years) at the INS for future research. Subjects will have the option to agree for this long-term storage in the ICF. Additional ethical approval will be requested if the samples will be used for screening of other pathogens or antibodies or for future research in this scientific field. If it

would be needed to ship samples to other countries, this will first be requested in appropriate MTAs and in a protocol amendment or addendum.

5.4.2 Laboratory testing

Diagnostic testing of specimens (both swabs and DBS) will be handled at the INS laboratory facilities in Marracuene in a BSL-2 laboratory using Standard Precautions. Viral inactivation (addition of the lysis buffer) of swab material will be performed in a certified Class II Type A1 or A2 biosafety cabinet during which full personal protective equipment (PPE) will be worn, such as a surgical mask, a face shield or goggles, two-pairs of gloves and an impermeable gown.

The total blood volume that will be collected for each individual participating in the serosurvey is 450µl by fingerprick and this will be repeated in each serosurvey round. This volume of blood will be collected on six circles of the DBS filter paper. Each of these circles takes approximately 75ul.

A. RT-PCR for sars-Cov-2 viral RNA detection

All collected nasal swabs will be checked for the presence of SARS-CoV-2 by real time reverse-transcriptase PCR (as described in Corman et al 2020). Depending on the prevalence in the population, RNA will be extracted from individual or pooled swabs (max 5 swabs per pool) using the available RNA extraction kit. Real-time reverse-transcription PCR will be performed under the conditions described in Corman et al 2020.

B. Luminex for anti-sars-CoV-2 antibody detection

Blood spot filter papers will be prepared by punching two discs of 4-mm diameter, and eluted overnight in 160 µL of PBS-TBN (dilution 1:40, PBS-1 % BSA-0.15 % Tween, pH 7.4, Sigma-Aldrich). Just before use in the immunoassay, the eluted samples will be further diluted to 1:200 in PBS-BN.

We will use an in-house developed multiplex antibody assay for the detection of anti-SARS-Cov-2 antibodies, similar to the protocol described by Ayouba A et al, 2020. As a target for the detection of anti-CoV antibodies in our Luminex platform, we have selected the main immunodominant antigens of the CoV family members: the large spike glycoprotein (S) and the nucleocapsid (NC) protein. We therefore coupled recombinant NC- and S-proteins of all currently known human CoVs (all bought at Sino Biological including SARS-CoV-2, INTERCHIM, Montluçon, France) to SeroMAP microsphere beads. These beads are carboxylated polystyrene microparticles that have been colour coded into 100 spectrally distinct sets or regions. Each of these regions can be quickly distinguished by an xMAP® Instrument, which allows the interrogation of up to 100 different analytes simultaneously from only a small volume of sample (<10µL). Biotinylated anti-human IgG, IgA and IgM will be used to determine positivity. A cut-off value will be estimated based on a panel of negative control samples also spotted on filter paper.

Similar procedures will be conducted for testing for antibodies to other coronaviruses.

Given that cross-reactivity with malaria can be expected (common in many serological assays), we will also test all field samples for malaria using a rapid diagnostic malaria test.

5.4.3 Long-term storage and destruction of specimens

INS will be the responsible entity for long term storage of samples. The temperatures of the freezer will be monitored by the INS's central monitoring system. Participants will be asked orally and in writing on the informed consent form to consent for long term storage of samples pertaining to the study (max 5 years after final study report) for future testing. Explicit informed consent will be requested for storage of samples for future testing to objectives not covered in this protocol. Before samples will be used, approval from the sponsor and the concerned ethics committees will need to be

obtained. If a participant refused long-term storage, his/her samples will be destroyed after the submission of the final study report. Specimens stored for long-term will not be destroyed but will remain in storage at INS laboratory until instructed by the PI and laboratory coordinator.

6. Safety Assessment

No investigational medicinal product will be administered or evaluated in this study, nor will it include invasive diagnostic procedures apart from the dried blood spot (finger prick) sampling for the purpose of the sero-survey. For this sampling, following a finger prick, a capillary blood sample will be applied on filter paper, to generate a dried blood spot. The blood volumes required for the study are less than 2% of the total blood volume of a healthy adult person and as such too small to affect the participants health. The participant might experience some discomfort from the finger prick: he/she may transiently feel dizzy or faint and could develop a bruise or swelling (and very rarely, a local infection) at the finger prick spot.

Respiratory specimens will be collected for diagnosis of SARS-COV-2 infection by PCR during the biweekly visits as part of the surveillance. Taking these respiratory samples could feel uncomfortable but entail negligible risk to the participants health.

Establishing a Data and Safety Monitoring board (DSMB) is not applicable for this study since no investigational product or intervention will be tested.

7. Statistical Analyses

7.1 Methods

All estimated transmission or clinical outcome indicators will be adjusted for the design effect, related to the selection of HDSS enumeration areas (clusters) within the source populations. Intraclass correlation coefficients can be deducted from previous studies in the HDSS. The results of the sero-survey and social mixing survey will additionally be adjusted for age-stratified sampling.

We will calculate a incidence rate of COVID-19 disease throughout one year, adjusting for the sensitivity of SARS-CoV-2 RT-PCR on nasal swabs by delay between symptom onset and testing. Using the longitudinal sero-survey data, determining the proportion of asymptomatics, we will be able to adjust the clinical incidence rate to a SARS-CoV-2 infection incidence rate, and determine the SARS-CoV-2 infection annual attack rate and the infection fatality risk by age group.

We will undertake three risk factor analyses: of SARS-CoV-2 infection, of COVID-19 disease (symptomatic infection), and of COVID-19 hospitalisation and death (severe disease). Using negative binomial regression, we will investigate the association with socio-demographic factors, clinical characteristics such as underlying medical conditions, and uptake of preventive measures. We also investigate the association between SARS-CoV-2 infection (from the sero-survey) and recent exposure, contact and mobility patterns (from the social mixing questionnaire).

We will assume everyone will be susceptible initially and basic and effective reproductive numbers (R0 and Re) will be calculated by the rate of change of incidence over successive serial intervals (time between onset of infection of index and onset of infection of infected).

We will use established methods (Cauchemez et al 2004; te Beest et al 2013) to estimate generation time distribution, ratio of transmissibility between symptomatic and asymptomatic cases, duration of

the infectious period, and the basic reproduction number, from the transmission data collected in households.

We will include heterogeneity in these parameters as far as possible, e.g. focus on the difference in transmissibility between children and adults. How much heterogeneity can be included in the estimation procedure will depend on the quality of the collected data. The estimation procedure will provide an estimate of the basic reproduction number, possibly composed of within household transmission and between household transmission, depending on the observations. These parameter estimates will be used to inform a compartmental model that describes the spread of SARS-CoV-2 on the population level. We have developed such models for the situation in the Netherlands (Teslya et al 2020) and are currently extending this modelling approach to include age structure of the population. The model can be fitted and/or validated using reported numbers of cases on the national level and estimates of attack rates in the population obtained from the repeated sero-surveys. We will develop scenarios of possible interventions in collaboration with local partners from Mozambique and use our models to project the possible impact of such interventions when rolled out in the population.

We have also developed a modelling approach that allows an assessment of the impact of social distancing and contact tracing on the doubling time of an outbreak (Kretzschmar et al 2020). This modelling approach was built on earlier work in modelling of outbreak response (see Kretzschmar et al 2004; Bonacic Marinovic et al 2014; 2015). In this work we have developed a framework to quantify timeliness of response based on disease specific parameters such as the generation time interval and proportion of secondary infections that are generated before symptom onset by an infectious case. We will explore whether data on contact tracing from Mozambique can be used to assess its impact on epidemic spread, and to derive guidance on how timely and complete tracing of contacts has to be so that it can be used as a tool to slow down or even contain the epidemic.

We will derive brand-specific total vaccine effectiveness against infection and against disease, in respectively a case-cohort design and a cohort design. Total vaccine effectiveness is the protection of vaccinees due to direct effects and added protection of vaccinees owing to vaccination of their neighbors or contacts, while overall vaccine effectiveness considers protection of an entire population, irrespective of the vaccination status of its individual members, due to the combination of total protection and protection of non-vaccinees due to vaccination of their neighbors or contacts (Ali et al 2015). To derive total vaccine effectiveness against infection, we will compare the proportion of sero-survey participants who seroconverted in the past 3 months among vaccinated against the proportion in non-vaccinated, adjusting for age and sex and other confounders. To derive total vaccine effectiveness against disease, we will compare the odds of vaccination among symptomatic confirmed SARS-CoV-2 cases detected through the population-based surveillance with the odds of vaccination among sero-survey participants who did not seroconvert in the past 3 months, in a case-cohort design. We will also explore overall vaccine effectiveness against disease using regression models comparing housing blocks with different levels of vaccination coverage among sero-survey participants.

We will estimate the conventional data-based secondary attack rate, which is the proportion of seroconversion among identified household contacts. However, these are not necessarily all secondary infections from the household index case, and could be tertiary. Therefore we will also derive a model-based secondary attack rate, estimating the number of actual secondary infections of the household index case. To do so, we could use a chain-binomial statistical model, as described by Jing et al (doi:10.1016/S1473-3099(20)30471-0), with an expectation-maximisation algorithm to account for (a) uncertainty in the infection time of asymptomatic infections, for (b) an incubation and infectious period distribution, as described in literature, and consider (c) the timing of symptom onset in

symptomatic household contacts and (d) the age-specific proportion of asymptomatic infections as estimated from the active population-based surveillance and sero-survey.

7.2 Sample size and power

Assuming a 40% annual attack rate, 50% symptomatic infections (cases), 2% overall case fatality risk, of which 90% are identified by the active population surveillance, 2790 COVID-19 cases and 56 deaths will be identified through the study period. The average incidence rate of the HDSS population would then be 1.50 cases per 100 person months with lower and upper 95% confidence interval limits 1.45 and 1.56. The overall case fatality risk would have as lower and upper 95% confidence interval limits at 1.53 and 2.58%.

To determine the infection attack rate from a simple random population sample of three age strata (comparable in size), with +/-3% confidence limits, 95% confidence interval, assuming the attack rate may reach 40% towards the end of the study period, samples from 800 individuals per age stratum per round will need to be collected.

Assuming the household secondary attack rate to be close to 17% (Fung et al, Clin Infect Dis, 2020) and a median household size of 7.3 (based on HDSS data), a 5% confidence limit and a design effect of 3, 642 household members will need to be enrolled, or 100 households. The WHO also recommended to include 100 households in household transmission investigations.

Assuming 20% vaccination coverage among the 2400 sero-survey participants, 5% of sero-conversion over 3 months and 10% of seroconversion over 6 months, a 95% two-sided confidence interval and an anticipated crude total vaccine effectiveness of 50%, there will be 66% power to observe this effect after 3 months and 97% power after 6 months.

8. Monitoring And Quality Assurance

Study monitoring and auditing will be performed in accordance with the sponsor's procedures, GCP guidelines and any other applicable regulatory requirements.

Upon approval of the protocol, the monitor will establish a monitoring plan. To ensure that the investigators and the study staff understand and accept their defined responsibilities, the monitor will maintain regular correspondence with the site and may be present during the course of the study to verify the acceptability of the facilities, compliance with the investigational plan and relevant regulations, and the maintenance of complete records.

Investigators and/or their study staff will be trained on the study protocol and all applicable study procedures prior to study initiation.

Study progress will be monitored by the CTU of the INS in collaboration with the CTU of the ITM as frequently as necessary to ensure the rights and well-being of study participants are protected; to verify adequate, accurate and complete data collection; protocol compliance and to determine that the study is being conducted in conformance with applicable regulatory requirements. Arrangements for monitoring visits will be made in advance in accordance with the monitoring plan, except in case of emergency.

From the electronic data, a weekly data collection report will be generated (using a Rmarkdown script), which allows verifying the completeness and accuracy of the data (the number of non-responders, missing data, compare the interviewed population's demographics with those of the source population, compare results from different interviewer teams). These reports will be used by the study monitor to strengthen the quality of data collection and entry. If the data quality of specific variables

or observations cannot be guaranteed, the epidemiologist with the support of a statistician could jointly decide to exclude specific observations and/or variables, with a justification for each exclusion (to be kept in the investigator's file and to be described and justified in the study report).

Beyond the qualifications of HDSS interviewers, HDSS data collection supervisors are expected to manage all team activities, deadline accomplishments, data quality control and assurance, retrain the other field staff and support them.

The study may also be subject to inspection by regulatory authorities (national or foreign) as well as the International Ethics Committees/Institutional Review Boards to review compliance and regulatory requirements.

9. Data Management

Data Management will be performed by the data manager at the ITM in collaboration with the data manager at INS and with the study staff at the sites involved in collecting and handling the study data.

A separate Data Management Plan will be prepared, with following essential data management aspects:

Data collection, handling and retention

Demographic, health and medical data from participants and households will be collected via electronic questionnaires on smartphones and/or tablets into the AfricoVER study database, by using Open Data Kit (ODK) and/or REDCap. The Open Data Kit is an open source software for collecting, managing, and using data in resource-constrained environments. Data collection will be done with *ODK Collect*, an open source Android app that replaces paper forms and is used in survey-based data gathering. REDCap (Research Electronic Data Capture) is a software widely used in the academic research community for building and managing surveys, databases and research studies. It is a secure web application as well as mobile application that can be used to collect, clean and manage subjects' information in accordance with various standards and applicable regulations (GCP, CFR 21 part 11, HIPAA). Furthermore, the software features amongst others a query management system and capabilities for importing and exporting data in various forms.

Paper questionnaires might be used if deemed necessary (e.g. as a backup when having issues with the electronic device). Only data defined by the study protocol will be collected. Both ODK and REDCap allow for offline data collection at remote sites. When connecting to the internet or cellular networks, data from the mobile device can be synchronized with the study server where the study data will be retained.

Edit checks and branching logic, programmed onto the electronic forms will validate at the point of data entry and support quality data.

Questionnaires will be tested and validated before household visits start. The INS data collectors, data collection supervisors, the data manager and the QA/QC team will be trained on informed consent procedures, data collection and entry before the study start.

Data security and confidentiality

The data management/IT system that will be used includes a robust security architecture including encryption, firewalls, antivirus software and controlled user access (smartphone authentication,

username, personal password and authorized user role). A list with all users who have access to the system will be kept updated during the study.

A backup of data will be provided at the server, computer, mobile device and/or database level where applicable or feasible.

The ITM and INS will also see to it that the necessary measures are taken to ensure that all data management documents (The AfriCoVER Data Management Plan, procedures, completed questionnaires) and IT devices and equipment (smartphones, tablets, computers, server) are kept secure (closed cupboards, closed and/or badge controlled offices and/or rooms).

Private data of the study participants will be handled confidential. Information such as the participant name or any other data which could lead to the identification of the participant will not be recorded in the study database(s) or on electronic transcripts, nor on any other paper documents (apart from the informed consent form) or electronic files. Household addresses, geographic coordinates of households, participant name and other contact data for each household or participant will be kept separate and limited to authorized staff at the study site(s) only. Instead, household addresses, participant names and contact data will be replaced by a specific study household and subject identification code (=pseudonym). These pseudonymized data will be used during the study.

Data sharing

AfricoVER will make its research data available for secondary research or to support the Mozambican COVID-19 response and by considering following essential aspects:

- Ensuring data confidentiality by providing data anonymized and unlinkable (amongst others by replacing the study household and subject identification code with a new code; by generalizing and randomizing specific variables).
- Making the AfriCoVER research data, which are in principal medical, personal and sensitive, available through a governed data access mechanism and along a transparent decision-making process, with: (i) Completion of a data request form, (ii) Evaluation by a data access committee, (iii) Data sharing Agreement, (iv) Secure transfer of anonymized data, and (v) Additional metadata which clarify the research data. Metadata will include the study protocol, questionnaire(s), and data dictionary.
- Ensuring that ethical permissions have been obtained by ethical committees and by study participants.
- Adhering to the FAIR data principles to ensure that research data are ‘findable’, ‘accessible’, ‘interoperable and ‘re-usable’.
- Providing data available through a repository for research datasets, which has sustainability planning in place to ensure long-term secure storage and availability.
- Sharing of interim and final research data as soon as possible to essential stakeholders involved in response and research, including public health institutions and research communities.

For data sharing between INS, the work package 2 (surveillance) lead, and ITM, the project coordinator, a Data Transfer Agreement has been signed by both parties. Transferred data will follow the same principles as mentioned above, but will be pseudonymized (personal information that could allow identifying a participant will be removed) instead of anonymized, which is essential for the data analysis following the study objectives.

10. Ethical Issues

10.1 Ethical and regulatory review

The study will be carried out according to the principles stated in the World Medical Association's Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects) as amended in 2013, all applicable regulations and according to established international scientific standards.

The study protocol will first be submitted simultaneously to the Institutional Review Board (IRB) of the Instituto Nacional de Saude (INS), to the IRB of the Institute of Tropical Medicine and to the Ethical Committee (EC) of the University Hospital Antwerp. Once approved it will be submitted to the National Committee for Bioethics in Health of Mozambique (CNBS; Comité Nacional De Bioética para Saúde). No participants will be enrolled in the study, nor will any study specific and subject related activities be performed before written approval is obtained from the IRB of ITM and INS and the national ethics committees in Mozambique and Belgium, the sponsor's resident countries.

Any subsequent study analysing the data or samples collected during AfriCoVER, will need to have a separate study protocol approved from by the ethics committee of the study principal investigator or sponsor, and from the CNBS in Mozambique. As mentioned in the section 'data sharing', this is also a requirement for sharing the research data collected during AfriCoVER.

10.2 Patient and/or community involvement

The HDSS community advisory board is being consulted on study procedures (eg. how and where to organise specimen collection), which has been successful and beneficial during previous studies integrated within the HDSS. Before the study starts, the HDSS staff will present the study to community leaders.

As soon as data are available and analysed after 3, 6 and 12 months, preliminary findings (COVID-19 incidence rate; case fatality; evolution of the reproduction number) will be presented to the HDSS community advisory board, health authorities and the national COVID-19 response team. Three months after evidence of local transmission, a larger preliminary analysis, including a first estimate of the infection attack rate and risk factor analysis will be presented to the same stakeholders, but also to community leaders.

10.3 Protocol amendments

Once the final clinical study protocol has been issued and signed by the authorized signatories, it cannot be informally altered. Protocol amendments have the same legal status and must pass through the appropriate steps before being implemented. Any substantial change must be approved by all the bodies and EC's that have approved the initial protocol, prior to being implemented, unless it is due to participant's safety concerns (in which case the immediate implementation can be necessary for the sake of participant's protection. In case modifications to the protocol or amendment are requested by any local EC during the review process, these must be discussed and agreed upon with the Sponsor prior to any resubmission incorporating those changes.

10.4 Informed consent

No participant may be admitted into the study until the Investigator or designee has obtained the written informed consent form.

10.5 Confidentiality

See "Data security and confidentiality" in section "9. Data Management"

The Data Protection Officer at the ITM will review the data protection measures taken within this study.

10.6 Risks and benefits

The minimal risks related to participating in this study are those related to blood and respiratory sample taking for laboratory testing: the discomfort of a finger prick and a nasal swab. The volume of blood removed will be too small to affect the participants health. Having biweekly household visits may be experienced as cumbersome. We do not know yet whether there may be potentially some risk of stigma if home testing in PPE will be done, even though consultations and sensitization with the community advisory board has already started before protocol development. A regular feedback loop with the community advisory board will be used as safeguard.

No antiviral drugs or any other treatment will be provided as part of this study and this study will not have direct influence on clinical outcome nor treatment. However, by participating in this study the participants health status will be closely monitored and the study participant will be referred to clinical care if needed. Participants are encouraged to inform the study team by phone if a household member develops clinical signs indicative of COVID-19. Those phone calls can be reimbursed, providing airtime allowing a 10 minute phone call, when a study staff member subsequently visits the household.

The risk of breaches of privacy and confidentiality breach will be kept at an ultimate minimum by not mentioning the participants name from processed samples nor in the study databases.

10.7 Compensation for participation

There will be no compensation for participation in this study.

10.8 Insurance

As required by the Belgian law on experiments on the human person of May 7th 2004, the sponsor has obtained a no-fault liability insurance with Amlin Corporate Insurance covering any harm, injury or (material) damage which may occur to study participants and which may be directly or indirectly caused by their participation in the trial. The insurance provisions will also be mentioned during the informed consent discussion.

11. Dissemination of results, intellectual property

All study documents are provided by the Sponsor to the Investigators and his/her appointed staff in confidence. None of this material may be disclosed to any party not directly involved with the study, without written permission from the Sponsor.

The study will be registered in ClnicalTrials.gov before recruitment of the first study subject. At the end of the study, a comprehensive study report will be prepared by the ITM in collaboration with the INS. This to serve the demands of funder, regulatory and ethical bodies when needed. Outcome data of this study will be entered in ClnicalTrials.gov and for access to other researchers.

Reporting and publication will be done in accordance with the CONSORT statement (http://www.consort-statement.org/consort-statement/). Results of this research (either positive or negative) will be rapidly communicated and the publication will be submitted for revision in open-access peer-reviewed journals. In addition, data will be made available as soon as possible for secondary research or to support the COVID-19 response. More details on data sharing are to be found in section 9 on Data Management.

12. Archiving

The sponsor and Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be verified. The relevant (essential) documents are those documents which individually and collectively permit to assess the conduct of the trial, the quality of the data produced and the compliance with GCP standards and applicable regulatory requirements. The Investigator's File should at least contain all the (essential) documents as listed in the procedure "Set up and maintenance of the Investigator Trial File". A copy of all source data and Case Report Forms must always be kept on site.

All the relevant study documentation present at all partners involved should be retained (in binders both at the site and at the ITM, except for the CRF that will be archived digitally at the ITM and on a USB at the site) for a minimum of twenty (20) and according to applicable local regulations. The Sponsor should be informed prior to destruction of the files.
After completion of the study, the IF will remain available for internal audits and/or inspections of regulatory authorities for a period of twenty years, unless differently requested by national authorities.

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