CLINICAL STUDY PROTOCOL

Streptococcus pyogenes carriage acquisition, persistence and transmission dynamics within households in The Gambia: a longitudinal cohort study

SCC No:	LEO 24005
Alias	SpyCATS (**S**treptococcus **PY**ogenes **C**arriage **A**cquisition, persistence and **T**ransmission dynamics within households in The Gambia: a longitudinal cohort **S**tudy
Other Number(s)	
Protocol Version – Date	Version 2.0 18th April 2022
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Signature page

The clinical trial will be carried out in accordance with the protocol, the ICH Harmonised Tripartite Guideline for Good Clinical Practice, and in accordance to local legal and regulatory requirements.

Principal Investigator:	**Signature:**	**Date:**
Edwin P Armitage		18 Jan 2021
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List of abbreviations

AE	Adverse Event
CRF	Case Report Form
CSD	MRCG Clinical Services Department
CSF	Cerebrospinal fluid
DMC	Data Monitoring Committee
GAS	Group A streptococcus
GCP	Good Clinical Practice
HIC	High-income countries
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
LMIC	Low- and middle-income countries
MDA	Mass drug administration
MRCG at LSHTM	Medical Research Council Unit The Gambia at London School of Hygiene & Tropical Medicine
MV0, MV1 etc.	Monthly visit 0, Monthly visit 1 etc.
PI	Principal Investigator
qPCR	Quantitative polymerase chain reaction
RHD	Rheumatic Heart Disease
WGS	Whole genome sequencing
WHO	World Health Organization

Protocol summary

Title:	*Streptococcus pyogenes* carriage acquisition, persistence and transmission dynamics within households in The Gambia: a longitudinal cohort study
Alias :	SpyCATS
Population:	Individuals within households in Sukuta
Number of participants:	45 households (approximately 450 individuals)
Number of Sites:	Household-based field study
Location of Sites (including satellite sites):	Field study in Sukuta Clinical Services Department MRCG Fajara Laboratories at MRCG Fajara
Study Duration:	18 months
Duration for Participants:	12 months
Objectives:	Primary: 1. To determine the prevalence, incidence, duration and transmission dynamics of asymptomatic GAS carriage and clinical GAS infections within households. 2. To establish risk factors for pharyngeal and skin clinical GAS infection, including detailed characterisation of the relationship with individual and household asymptomatic carriage, *emm* type and seasonality. 3. To develop a mathematical model of household GAS transmission using clinical, behavioural and phylogenetic relatedness data to calibrate it, to allow for estimation of the relative contributions of between and within household transmission. Secondary: 1. To determine risk factors for asymptomatic GAS carriage. 2. To describe the relative importance of asymptomatic GAS skin and throat carriage in this setting. 3. To describe any seasonal variation in GAS carriage and clinical GAS infection throughout the year. 4. To describe GAS emm type diversity in this setting. 5. To investigate the extent of GAS tissue tropism of emm types identified. 6. To determine the prevalence and incidence of groups C and G streptococcal carriage and clinical infection in this setting. 7. To describe variations in bacterial density by site, season and clinical characteristics using quantitative PCR.

8. To describe anti-GAS seroprevalence and investigate serological responses to asymptomatic GAS carriage and clinical GAS infection.

9. To identify non-human reservoirs of GAS within households and the presence of airborne GAS indoors using settle plates.

10. To describe the antimicrobial sensitivity of GAS isolates identified.

11. To explore GAS-specific serological and mucosal immune activity in response to GAS colonization and disease, and to identify correlates of protection.

Endpoints:

Primary endpoints:

- Asymptomatic GAS throat and skin carriage and clinical GAS infection prevalence at each monthly visit
- Asymptomatic GAS throat and skin carriage and clinical GAS infection incidence per person year
- Duration of asymptomatic GAS throat and skin carriage to the nearest week
- Phylogenetic relatedness of GAS isolates by whole genome sequencing
- Clinical examination findings and features of clinical GAS infection

Secondary endpoints:

- Presence of risk factors for GAS carriage or disease at individual and household levels
- Seasonal variation in GAS carriage and infection prevalence
- GAS *emm* type
- Groups C and G streptococcal prevalence and incidence
- Quantification of bacterial presence by qPCR
- GAS serological responses (including seroprevalence and fold-change in antibody titres in response to asymptomatic carriage and clinical infection).
- GAS presence on household swabbing and settle plates
- GAS antimicrobial sensitivity

Description of Study Design:

A prospective cohort study within households in Sukuta, The Gambia, will be conducted. A total of 45 households will be recruited, including every household member as individual participants (resulting in approximately 450 participants).

Participants will be enrolled for 12 months, covering one full rainy season, undergoing an enrolment visit (MV0), then monthly visits (MV1, MV2, MV3, etc. until MV12). At each visit an oropharyngeal and normal skin swab, a saliva sample and

a dried blood spot (DBS) will be taken, data collected on socio-demographics, social-mixing/behavioural factors and clinical examination findings. Swabs will also be taken from any pyoderma lesions, environmental swabs from common touch points and settle plates used inside households. In addition, a blood for a serum sample will be taken at the enrolment and final visits.

A subgroup of 16 random households, will undergo more frequent (weekly) throat and skin swabbing for a period of 6 weeks, during the second 6 months of the study.

Weekly swabs will also be taken from participants who become GAS (or GCS/GGS) carrier positive within the first 6 months, until they have had 2 negative swabs.

Participants reporting symptoms suggestive of possible clinical GAS infection such as pharyngitis or pyoderma (but also including invasive infections), will be visited for an unscheduled visit where a clinical and health-seeking history will be recorded, a swab, a saliva sample and a DBS taken, and treatment (or further management) will be delivered according to WHO guidelines.

Swabs from participants will be cultured for the presence of GAS (and GCS/GGS) and isolates will undergo whole genome sequencing (WGS) in order to assess phylogenetic relatedness of strains identified. These data, combined with temporal incidence of strain acquisition, clinical data and household situation will be used to build a mathematical model of GAS transmission to identify key routes of transmission within and between households in The Gambia.

1 Background information and rationale

1.1 Background information

Group A Streptococcus (Streptococcus pyogenes, GAS) is a beta-haemolytic Gram positive bacterium that is a major cause of infectious disease burden globally, responsible for over half a million annual deaths (1-4). It causes a wide spectrum of disease from superficial skin and pharynx infections through to invasive disease, in addition to the immunological sequelae of acute rheumatic fever, rheumatic heart disease (RHD) and acute post-streptococcal glomerulonephritis (3, 5). Each year an estimated 1.8 million invasive GAS, 111 million pyoderma and 616 million pharyngitis cases are thought to occur globally (2). The majority of clinical GAS infections are thought to occur in low-income countries, though the data from such countries is the most lacking (1, 2, 6, 7).

Rheumatic heart disease, the most serious immunological consequence of GAS infection, causes over 300,000 deaths each year, predominantly in low- and middle-income countries (LMIC) (3). RHD surveillance data from Africa are lacking, prevalence estimates in sub-Saharan Africa (sSA) range between 7.4 and 51.6 per 1000 children (8). Ethiopian children with RHD were shown to have a 12.5% annual mortality rate (9).

Despite the significant burden of disease caused by GAS and its immunological sequelae globally and particularly in LMIC such as The Gambia, data are limited on interventions to reduce GAS carriage, transmission and associated disease in LMIC (10, 11). Furthermore, the understanding of the natural history of GAS carriage, transmission and infection is limited. An understanding of carriage duration, sociodemographic and behavioral factors affecting carriage and transmission, seasonal variation, the relationship between carriage and disease, and transmission patterns within households are all crucial in order to design and implement interventions targeting GAS in LMIC. The limited understanding that we have on these aspects of GAS are mostly from high-income countries (HIC) (12-14). In LMIC, higher prevalence and incidence of GAS carriage may underlie the higher burden of GAS-related clinical infections and immune sequelae seen (1-3).

The epidemiology of superficial GAS infections in The Gambia is poorly understood, therefore in 2018 we conducted a cross-sectional study in Sukuta in 1441 children under 5 to determine the prevalence of common skin infections including GAS pyoderma and scabies, which is important in pyoderma prevalence due to the common presence of bacterial co-infection of scabies lesions. We showed a high prevalence bacterial pyoderma (17.4%), and scabies infestation (15.9%), and specifically of GAS culture-positive pyoderma (8.8%). We also found a significant increase in pyoderma during the rainy season (before the start of the rains vs. after: 8.9% vs. 23.1%, aPR 2.42, CI 1.39-4.23).

Whole-genome sequencing (WGS) has transformed our ability to understand GAS epidemiology, giving significant resolution to determine linkage between strains. WGS has been critical in GAS outbreak investigations in HIC (15-18), but has not be used in African settings. Combining phylogenetic relatedness data from WGS, clinical and behavioural data with mathematical models can provide new insights into transmission dynamics and potential intervention strategies, and has not been attempted for GAS in any LMIC setting before.

References of literature and data are listed in Section 14.

1.2 Rationale

Despite the significant burden of disease caused by GAS in LMIC, prevention and treatment of GAS disease is lacking. In HIC, superficial GAS infections such as pharyngitis and pyoderma (impetigo) are typically diagnosed early and often treated with antibiotics, within well-developed primary healthcare systems. In low-resource settings, where living conditions and environment often put people at higher risk of GAS infections, and primary healthcare is more limited, a different model of care is required to tackle the burden of GAS disease. While effective vaccines against GAS have not yet been developed, mass drug administration (MDA) of scabicides such as ivermectin, in areas where scabies is a major driver of GAS, have been shown to be an effective method at reducing GAS infections for a prolonged period of time following a single administration of MDA, and could be increasingly used in low-resource settings (19-21).

Whether MDA directly targeting GAS, for example with Azithromycin, might be an effective strategy is unknown at this time. Theoretically, MDA might be effective in reducing GAS infections in a population due to treatment of active GAS infections reducing the pool of contagious individuals, and thus reducing the risk of transmission of GAS from an infected person to a non-infected person. However, it might also be effective at reducing the rates of asymptomatic carriage of GAS in the population, and thus reducing spread of GAS from asymptomatic carriers to non-infected people and non-carriers. WGS of GAS isolated from The Gambia showed a much higher *emm* type diversity than in HIC, suggesting that infection acquisition may be related to widespread carriage and transmission rather than highly clonal large single-strain outbreaks as seen in HIC (17, 18, 22). The role of asymptomatic pharyngeal carriage and non-pharyngeal reservoirs of GAS such as skin and environmental sources are not well understood.

To understand if MDA might be effective at reducing GAS outside of the context of scabies control, and to better understand how, when and where MDA should be used in future in regions such as The Gambia, it is essential to understand the natural history of GAS carriage, persistence, transmission dynamics and clinical infection. We have shown a high level of GAS skin infections in The Gambia in a cross-sectional study, but in order to understand the temporal relationship between carriage and disease, how GAS is spread from person to person, via skin or pharynx, acquired inside or outside households, and other risk factors for carriage and disease, a longitudinal study is essential. Very few longitudinal studies of GAS exist (12, 23), and high quality data from high-prevalence countries in sub-Saharan Africa combining classical epidemiology with detailed social-mixing behaviour and next generation WGS techniques to model disease transmission will be highly informative in growing our understanding of GAS epidemiology.

2 Study objectives

Primary:

1. To determine the prevalence, incidence, duration and transmission dynamics of asymptomatic GAS carriage and clinical GAS infections within households.

2. To establish risk factors for pharyngeal and skin clinical GAS infection, including detailed characterisation of the relationship with individual and household asymptomatic carriage, *emm* type and seasonality.

3. To develop a mathematical model of household GAS transmission using clinical, behavioural and phylogenetic relatedness data to calibrate it, to allow for estimation of the relative contributions of between and within household transmission.

Secondary:

1. To determine risk factors for asymptomatic GAS carriage.
2. To describe the relative importance of asymptomatic GAS skin and throat carriage in this setting.
3. To describe any seasonal variation in GAS carriage and clinical GAS infection throughout the year.
4. To describe GAS *emm* type diversity in this setting.
5. To investigate the extent of GAS tissue tropism of *emm* types identified.
6. To determine the prevalence and incidence of groups C and G streptococcal carriage and clinical infection in this setting.
7. To describe variations in bacterial density by site, season and clinical characteristics using quantitative PCR.
8. To describe anti-GAS seroprevalence and investigate serological responses to asymptomatic GAS carriage and clinical GAS infection.
9. To identify non-human reservoirs of GAS within households and the presence of airborne GAS indoors using settle plates.
10. To describe the antimicrobial sensitivity of GAS isolates identified.
11. To explore GAS-specific serological and mucosal immune activity in response to GAS colonization and disease, and to identify correlates of protection.

2.1 Study endpoints

Primary endpoints:

- Asymptomatic GAS throat and skin carriage and clinical GAS infection prevalence at each monthly visit
- Asymptomatic GAS throat and skin carriage and clinical GAS infection incidence per person year
- Duration of asymptomatic GAS throat and skin carriage to the nearest week
- Phylogenetic relatedness of GAS isolates by whole genome sequencing
- Clinical examination findings and features of clinical GAS infection

Secondary endpoints:

- Presence of risk factors for GAS carriage or disease at individual and household levels
- Seasonal variation in GAS carriage and infection prevalence
- GAS *emm* type
- Groups C and G streptococcal prevalence and incidence
- Quantification of bacterial presence by qPCR
- GAS serological responses (including seroprevalence and fold-change in antibody titres in response to asymptomatic carriage and clinical infection).
- GAS presence on household swabbing and settle plates
- GAS antimicrobial sensitivity

3 Study design

3.1 Type of study and design

The proposed study is a prospective, longitudinal (open) cohort study within households in Sukuta, The Gambia. Households will be recruited, and all household members present at the time of the visit will be asked to participate. Households will be followed for 12 months, with monthly visits, and more frequently for some subgroups of participants.

A total of 45 households will be recruited, including every available consenting household member as individual participants (resulting in approximately 450 participants with the average household size being around 10 in Sukuta).

Households will be enrolled for 12 months, with enrolment aiming to commence before the rainy season, to ensure that the enrolment period will span one full rainy season, with dry periods either side. Every household will undergo an enrolment visit (MV0), then monthly visits (MV1, MV2, MV3 etc., up to MV12) or less frequently if practical constraints arise. At each visit, the household size will be determined by the number of individuals who slept in the household the previous night, and those household members present will be asked to participate. Household members not available to be seen will be still allocated a household ID number, in order to capture relevant information regarding their social mixing with other household members, and if they are present at later visits, they will be asked to enrol. Participants who's baseline (enrolment) visit occurs after MV0 will be asked why they were not available previously. Reasons for missed visits and late enrolment will be captured.

At each visit an oropharyngeal (OPS), normal skin swab (NSS), oral fluid (OF) and dried blood spot (DBS) will be taken from all individuals present, data collected on socio-demographics, behavioural factors and clinical examination findings. In addition, a blood sample for serum (BS) may be taken at the beginning and end of the study for detailed functional immune responses. This may be done by arranging a clinic visit if more appropriate. Swabs will also be taken from any pyoderma lesions, from common touch points in the household such as door handles, and settle plates (SP) used inside households. Throughout the study, if an enrolled participant reports symptoms consistent with GAS infection, they will have an unscheduled visit including a physical examination an appropriate swab (oropharyngeal or pyoderma), OF and DBS. Swabs will be taken the same day to the research microbiology laboratory and plated for culture. The culture will identify the presence of GAS (and Groups C and G Streptococcus) from the swabs, antibiotic resistance will be determined and any GAS (and GCS and GGS) isolates will be stored for later use. Skin and pyoderma swabs will also be cultured for *Staphylococcus aureus* and isolates stored for later use. Additional clinical samples will be taken at various time-points including serum blood samples, dried blood spot and salivary samples to contribute to exploratory objectives.

Swabs from participants will be cultured for the presence of GAS and isolates will undergo whole genome sequencing (WGS) in order to assess phylogenetic relatedness of strains identified. These data, combined with temporal incidence of strain acquisition, clinical data and household situation will be used to build a mathematical model of GAS transmission to identify key routes of transmission within and between households.

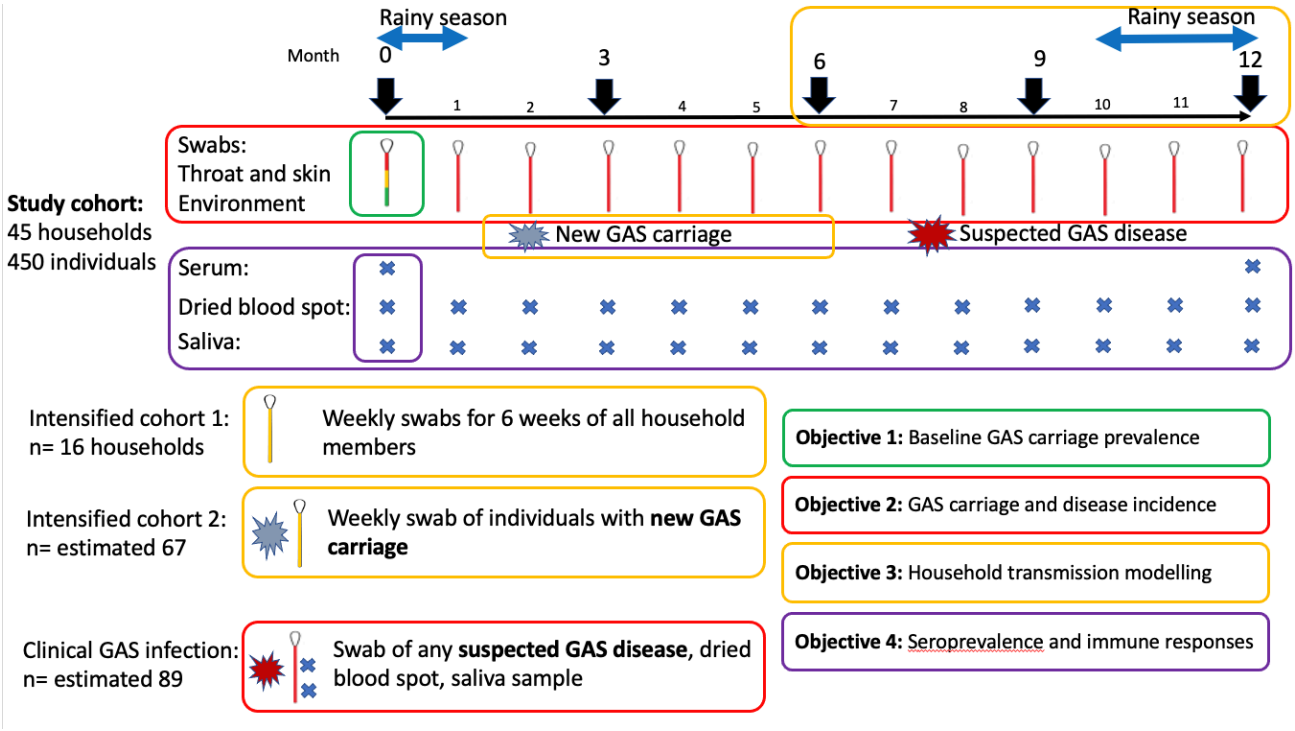


Figure 1. Visit and sampling schedule schematic

3.2 Sub-studies

3.2.1 Intensified incident GAS surveillance cohort

A subgroup of 16 randomly selected households will form a cohort to undergo intensified swabbing. This cohort will be used to assess incidence and duration of GAS carriage and disease with greater resolution than the main cohort. All household members present will undergo more frequent swabbing (OPS and NSS), with a view to being visited every week for 6 weeks. At these visits, more detailed social-mixing behaviour data will be collected. This more intensive temporal epidemiology with the social-mixing behavioural data collected will be combined with WGS data to inform the household transmission model.

3.2.2 Estimating duration of GAS carriage

A further subgroup will also be identified to allow for greater resolution in the estimation of the duration of GAS (and GCS/GGS) carriage. Following MV0, any participant who becomes GAS (or GCS/GGS) carrier positive (i.e. was negative at baseline or the previous visit, and then becomes positive at a monthly visit) will have weekly swabs taken from the same site that was positive, until 2 GAS negative swabs have been taken in a row.

3.2.3 Estimating GAS infection incidence

To assess incidence of clinical GAS disease in the cohort throughout the study, all participants reporting symptoms suggestive of possible clinical GAS infection such as pharyngitis or pyoderma (but also including invasive infections), will be visited for an unscheduled visit where a clinical and

health-seeking history will be recorded, a swab, saliva and DBS taken, and treatment (or further management) will be delivered according to WHO guidelines or best local practice. Furthermore, at each scheduled visit, any individual identified with symptoms of clinical GAS infection will undergo the same process of history-taking, swabbing, treatment and follow up.

4 Selection and withdrawal of participants

4.1 Selection of participants

The study will enroll participants as individuals within households. Households will be identified using a process of random selection. No complete sampling frame of households exists for Sukuta, however GIS data exist from the 2013 census of The Gambia. These data will be utilized to obtain a random set of sampling locations stratified by population density. A list of GPS coordinates for the locations will be identified and for each location, the nearest household will be approached for participation. Each location on the list will be approached in order until the desired sample size is reached. Households will only be enrolled if all members of the household consent to participate in the study.

For the purposes of enrolment in the study, a household will be defined according to The Gambia Demographic and Health Survey 2013 definition: “a household [is] defined as a person or a group of related or unrelated persons who live together in the same dwelling unit(s) or in connected premises, who acknowledge one adult member as the head of the household, and who have common arrangements for cooking and eating.”

4.2 Eligibility of participants

Households and all individual participants must meet all of the inclusion criteria and none of the exclusion criteria to be eligible to participate in the trial.

Participants must meet all of the inclusion criteria and none of the exclusion criteria to be eligible to participate in the trial.

4.2.1 Inclusion criteria

Households must:

- Be within the boundary of Sukuta as determined by the 2013 census
- Have at least 3 members including at least one child under age 18

Individuals must:

- Provide signed (or thumbprinted) informed consent for study participation (obtained from a parent or guardian for children under the age of 18
- Be willing and have capacity to participate and comply with the study protocol as judged by a member of the study team
- Be resident in the household, with no plans to move outside of the household during the period of study participation

4.2.2 Exclusion criteria

Households:

- Less than 50% of individuals living in the household, as defined by the The Gambia Demographic and Health Survey 2013 definition, provide consent to participate

Individuals:

- Consent not provided
- Has any condition or any other reason that may lead to difficulty or discomfort in obtaining all the necessary samples
- Is judged by the study team member to be unable or unlikely to participate and comply with the study protocol for the entire study period

4.3 Withdrawal of participants

A study participant will be discontinued from participation in the study if:

- Any clinical significant adverse event (AE), laboratory abnormality, intercurrent illness, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
- Development of any individuals' exclusion criteria.

Participants are free to withdraw from the study at any time without giving a reason. Household heads are free to withdraw their household from the study at any time without giving a reason.

If an individual withdraws consent to participate in the study, or their participation is discontinued by the study team for any of the above reasons, this will not necessitate the withdrawal of the other members of their household if the discontinuation occurs after the first month (once MV1 visits commence) of the study. If an individual withdraws within the first month (before MV1 visits commence), it may be determined by the PI that the entire household be withdrawn and another household recruited as described above. If not possible or practical to recruit another household, the household with a withdrawn member may continue in the study.

4.3.1 Missed visits and visit windows

Household visits will be arranged for the same day of the month as the enrolment visits where possible, to within 14 days. For example if a household's enrolment visit (MV0) is on the 5th of March, their M1 visit will be arranged for 5th April, unless this coincides with a weekend, in which it may be delayed or brought forward (within 14 days either way) to a working day. Households will be reminded by phone by the study team a few days in advance of a study visit. They will be reminded that all household members should be present if possible. All possible efforts will be made arrange the visit to be at a convenient time of day when all household members can be present. If the visit must be rearranged due to unexpected circumstances such as a funeral, the study team may rearrange the visit to another day (within 14 days of the required date where possible).

Where it is not possible to arrange a time when most household members are present, or if a household member is missed due to any other reason, the study team will attempt to collect the required samples from them within 7 days of the household visit where is it feasible.

For participants undergoing weekly visits for intensified swabbing, the visit will be arranged on any day of the next working week. If more than one weekend passes between weekly visits, then a visit will have been missed.

If a household or individual misses their visit and sampling windows, they will continue in the study according to all subsequent planned visits. Samples for the missed visit window will not be collected.

5 Study procedures and evaluations

5.1 Sensitization

Sensitizing potential study participants will precede the formal recruitment period to ensure that they are aware of the study as far in advance as is practical and therefore are given as much chance as possible to consider their potential involvement prior to providing informed consent. Sensitization will be approached using community and household/individual level strategies.

5.1.1 Community sensitization

Prior to any study recruitment, a meeting, or series of meetings, will be arranged with the village Alkalo and other key leaders within the community such as elders and community stakeholders to discuss the study, to sensitize the community to the study, and to answer any questions or concerns that may be raised. Details of the study will be provided by the study team and discussed, allowing for everybody present to gain full understanding of the purpose of the study, and the risks and benefits as outlined in the informed consent document. The study team will also ask the village leaders and stakeholders present to disseminate information about the study to the wider community.

5.1.2 Household and individual sensitization

Once a household has been identified from the GPS location on the potential household list (as described in section 4.1) members of the study team will approach the household to sensitize them to the study. At least two days in advance of the enrolment visit, the study team will approach the households to ask to speak with the head of the household. If they are willing, the study team will then sensitize the household head (and other household members present if they wish) to aspects of the study including the purpose, procedures, risks and benefits of participation. They will answer any questions that household members may have, and distribute copies of the informed consent document information sheet.

If the household and individuals meet the inclusion criteria, and the household head is potentially willing to participate, the study team will complete the sensitization log, and sensitization form with details of the household composition, including contact details. They will then make an appointment for and informed consent visit the following day (or another day at least 24 hours after the initial sensitization visit) and will then ask the household head to discuss the study with the whole household in advance of the next appointment, and come to a decision about whether the entire household would be willing to participate in the study.

5.2 Informed consent

At the informed consent visit (at least 24 hours after sensitization), the study team will discuss the study once again with the household head and other household members to confirm that they have understood the consequences of study participation and to answer any remaining questions. If all the inclusion are me and none of the exclusion criteria are, the study team member will proceed to obtain informed consent from all household members.

If at the consenting visit, the study team member determines that the household does not meet the inclusion and exclusion criteria, or anyone in the household is not willing to take part, then they will not proceed with the consent process.

Once the desired sample size of 45 households have been consented, study recruitment will be complete. If any household withdraws consent, or cannot participate for any other reason after consenting, they may be replaced by another household (the next GPS location on the list). Individuals who were not present at MV0 and subsequent visits but wish to participate at a later time may provide informed consent and enrol at any later monthly visit.

5.2.1 Informed consent for adults aged 18 years or more

To obtain informed consent from the household members, in the presence of a literate witness, a member of the study team will translate the informed consent document (ICD), which is in English, line-by-line into the local language spoken by the consenting individual (e.g. Mandinka, Wolof or Fula). Once the entire ICD has been translated, the study team member will answer any questions that the individual may have. If the consenting individual remain willing to participate and to provide informed consent, the study team member will document their informed consent on the signature page of the ICD. The study team member will document the participant's name, the name of the head of household and the language of consent. If the participant is literate, they will be invited to write the date and time and then sign the ICD. If the participant is not literate, the witness will write the date and time and the participant will be asked to thumb-print the signature portion of the ICD. The witness will then date and sign to confirm that the participant voluntarily agreed to take part in the study. The study team member will then write the date and time and sign the section relating to the person obtaining consent.

5.2.2 Provision of assent by children aged 12-17 years

For children aged between 12 and 17 years inclusive, they will be asked to provide assent to participate in the study in addition to the informed consent provided by the child's parent or legal guardian. In order to do so, the study team member will explain the study in simplified form to the child in an age-appropriate way, and will answer any questions that they may have. They will then be asked to sign (or thumbprint) the "participant's assent" section. The parent or legal guardian will also be required to sign (or thumbprint) the ICD form for the child, as described in section 5.2.1.

5.2.3 Informed consent for children aged less than 12 years

If the participant is under the age of 18, the child's parent will be required to sign (or thumbprint) the ICD on their behalf. When documenting consent for the child, one of their parents or a legal guardian will be asked if they provide consent for the child to participate undergoing the same process as described in section 5.2.1. The study team member will write the child's name on the ICD under "participant's name", then they will write the name of the parent or guardian providing consent under the relevant section.

5.2.4 Confirmation of ongoing willingness to participate

A certified copy of the signed ICDs for every participate will be provided to them at the enrollment visit. Written consent is obtained only at the consent visit, but opportunities to ask further questions will be given at each visit and ongoing willingness to participate in the study will be confirmed verbally at each opportunity. It will be made clear at every point, that participation is voluntary, and they may decline to participate or withdraw consent to participate at any point without giving a reason.

5.3 Study household visits

5.3.1 Visit planning and logistics

Enrolment for the study will aim to commence 1-3 months before the expected start of the rainy season in 2021. Prior to the enrolment visit, the study team will identify all of the consented households and schedule their enrolment visits in the most logistically practical way for the upcoming days and weeks in order to minimize travel, and evenly distribute the number of participants to be seen and sampled each day (Monday to Thursday). With 45 households to be seen within a month, and an average household size of 10, this will mean 2 to 3 household visits will be required each working day, with approximately 25 to 30 individuals seen each working day for the main cohort. Another team will perform the weekly visits for the intensified swabbing cohorts.

Prior to each visit day, the study team will call at least one household member, ideally the household head, to arrange the visit as scheduled, and to ensure that all household members will be present at the arranged time. If not possible that every household member is there, it may be necessary to go ahead with the visit without everyone present, but arrange for a study team member to reattend within the visit window to see the missing household member.

It may be necessary to move a visit at short notice due to public holidays, religious ceremonies, illness or other unforeseeable reasons. In such cases, the study team will make every effort to inform the study participants affected as soon as possible and to rearrange their planned visit (within the visit window – see section 4.3.1).

5.3.2 Enrolment visit (MV0)

At the enrolment visit (MV0), each individual will undergo a baseline survey including participant's sociodemographic data, medical history and social-mixing/behavioural factors. A physical examination will be carried out including a full body examination (taking care to maintain privacy) looking for any evidence of skin infections, and swabs will be collected from the oropharynx and from normal skin. Additionally, a salivary sample will be obtained, and blood will be taken for a serum sample and dried blood spot. Any individual who is exhibiting symptoms of pharyngitis (sore throat and pharyngeal inflammation) will be further examined and managed as they would be at an unscheduled visit for possible GAS infection (see section 5.3.4 below). Any individual who is identified as having evidence of pyoderma (bacterial skin infection involving pus or crusts), will have a wound swab taken, and will be provided antibiotic treatment according to WHO or local guidelines as appropriate. Any additional abnormal finding requiring further investigation or treatment will be managed according to local practice or an appropriate referral made according to the nature of the finding (see section 5.3.4).

Alongside the individual surveys, data will be collected for each household such as household setup, family relationships, sleeping arrangements, presence of mosquito nets, number of rooms, number

of buildings and access to water points. Environmental swabs will be taken from common touch points such as door handles and soft furnishings, and settle plates will be used indoors to investigate airborne transmission.

5.3.3 Monthly visits (MV1, MV2, MV3 etc. up to MV12)

Participants will be enrolled for 12 months undergoing an enrolment visit (MV0), then up to monthly visits (MV1, MV2, MV3 etc. up to MV12), though enrolment may occur at a visit later than MV0.

At and each monthly visit following enrolment, the study team will collect further survey data from each individual household member, collect an oropharyngeal and a normal skin swab, dried blood spot and salivary samples. Data collected will include updated socio-demographic information, social-mixing and behavioural factors and clinical examination findings. Any evidence of pyoderma will be swabbed and treated, and any other abnormal finding requiring further investigation or treatment will be managed according to WHO guidelines or best local practice or an appropriate referral made according to the nature of the finding. Additionally, any use of antibiotics or other medication, or any attendance at a healthcare setting since the previous visit will be recorded.

5.3.4 Unscheduled visits for possible GAS infection

At any point during a participant's enrolment in the study, they may develop symptoms consistent with a superficial GAS infection (acute pharyngitis or pyoderma), a severe GAS infection, or an acute reaction to a GAS infection such as acute rheumatic fever (ARF). This may occur, or be picked up, at a scheduled monthly or weekly visit, but could occur at any other time. If this occurs between scheduled visits, they will be asked to contact the on-call study nurse, who will take details of the complaint over the phone, and depending on the severity of the symptoms will arrange for the participant to be seen either urgently at the MRC Unit Clinical Services Department, or less urgently at their compound the next day (or as soon as reasonably possible). The study nurse will be able to consult with the PI or another delegated clinician for medical advice.

In the case of a non-urgent visit, the study nurse will collect data on the history of the complaint, any medication taken and other relevant information. They will perform a physical examination, including vital signs. If the symptoms are consistent with possible GAS infection (pharyngitis or pyoderma), then a study swab will be taken (throat or wound) for culture alongside a saliva sample and a dried blood spot test. Treatment will be provided for pyoderma according to WHO or local guidelines. For suspected pharyngitis, treatment will provided empirically based on WHO or local guidelines, and then reviewed following the culture result.

In the case of urgent visits at the Clinical Services Department, a study team member will attend the ward to collect this data as soon as possible, and take the study swab if applicable (ideally prior to any antibiotic therapy). Any further investigations performed and treatment given by the clinical services team will be recorded. In the case of suspected invasive GAS infection, the patient would be admitted for treatment. A blood culture should be performed as part of clinical assessment and if GAS is identified, the isolate will be obtained from the clinical microbiology laboratory for further analysis including whole genome sequencing. In the case of invasive GAS or suspected ACF the study team would obtain a separate serum sample.

5.3.5 Weekly visits for households in the intensified incident GAS cohort

A subgroup of 16 randomly selected households will be selected to participate in the intensified incident GAS surveillance cohort. These households will be visited up to every week for 6 weeks during the second half of the study, in addition to their regular monthly visits at MV1, MV2 etc. The

purpose of this cohort is to investigate asymptomatic GAS carriage incidence with greater resolution (more frequent swabbing) than the main cohort. At the weekly visits they will undergo OPS and NSS. Additionally these households will be asked more detailed questions about social-mixing behaviours such as school, mosque and market attendance frequency in the form of a daily diary or similar. These data will be used in combination with WGS data from any GAS identified to inform the household transmission model.

At these visits the study team will complete a survey for all household members containing a brief medical history and physical examination, and a more extensive social-mixing behaviours section. Throat and normal skin swabs will be taken, and any symptoms identified managed as above (section 5.3.4).

5.3.6 Weekly visits for asymptomatic GAS (or GCS/GGS) carriers

All participants not already in the intensified incident GAS cohort, who's OPS or NSS grow GAS (or GCS/GGS) on culture at a monthly visit following a previous negative swab, will be classified as "new carriers" who will then be swabbed weekly until such time as 2 negative swabs in a row have been obtained from the same site that was positive i.e. a participant who's OPS was positive at MV1 will have weekly OPS until negative twice in a row, whereas a participant with a positive NSS will have weekly NSS.

At these weekly visits, only the household member who is a "new carrier" will be seen and swabbed, not the other household members. The study team will ask a brief medical history, perform a physical examination, and take the swab from the previous positive site. If the participant has developed any clinician symptoms potentially in connection with their positive swab, then they will be treated as a GAS infection case as described in section 5.3.4.

5.3.7 Nested cross-sectional study of personal hygiene behaviour

At a one monthly visit, participants will be requested to undergo an additional survey on their personal hygiene behaviours for the last week including laundry, hand-washing, bathing and soap and disinfectant use. Attitudes towards wound-care and usual practives of participants in response to wounds will be captured.

At the same monthly visit, additional environmental swabs will be collected from the household including 4 commonly touched locations within the household and a sample of water from the main household greywater source. The swabs will be cultured as described in section 5.6.2.

These data, combined with individiuals' carriage and infection data from the wider study will be used to assess the relationship between individiaul and household-level hygiene behaviours and GAS, GCS/GGS and *S. aureus* carriage, infection and reservoir presence within households in this setting.

5.4 Study visit and sampling schedule

Table 1. Visit data and sampling schedule for the various cohorts *only at MV0 and MV12

Visit timing	Visit window	Data and samples	Main cohort	Incidence cohort (16 households)	Duration cohort (new carriers)
	-	Eligibility	X	-	-
		Sociodemographics	X	-	-

Month 0 enrolment visit (MV0)		Social mixing behaviour	X	-	-
		Household setup	X	-	-
		Medical history	X	-	-
		Physical examination	X	-	-
		Oropharyngeal swab	X	-	-
		Normal skin swab	X	-	-
		Blood serum*	X	-	-
		Dried blood spot	X	-	-
		Salivary sample	X	-	-
		Environmental swabs	X	-	-
		Settle plates	X	-	-
Weekly visits	+/- 7 days	Medical history	-	X	X
		Extended social-mixing behaviour	-	X	-
		Physical examination	-	X	X
		Oropharyngeal swab	-	X	(X) *if previously positive*
		Normal skin swab	-	X	(X) *if previously positive*
Monthly visits (MV1, MV2, MV3 etc. up to MV12)	+/- 14 days	Update sociodemographics	X	X	-
		Medical history	X	X	-
		Social-mixing behaviour	X	-	-
		Extended social-mixing behaviour	-	X	-
		Update household setup	X	X	-
		Physical examination	X	X	-
		Oropharyngeal swab	X	X	-
		Normal skin swab	X	X	-
		Blood serum*	X	X	-
		Dried blood spot	X	X	-
		Salivary sample	X	X	-
		Environmental swabs	X	X	-
		Settle plates	X	X	-
Unscheduled visits (may	-	Clinical history	X	X	X
		Physical examination	X	X	X

occur at scheduled visits if symptoms present)		Wound or throat swab	(X) *if applicable*	(X) *if applicable*	(X) *if applicable*
		Salivary sample	(X) *if applicable*	(X) *if applicable*	(X) *if applicable*
		Blood sample	(X) *if applicable*	(X) *if applicable*	(X) *if applicable*
Personal hygiene visit (done at another monthly visit		Personal and household hygiene questionnaire	X		
		Extended environmental swabbing	X		

5.5 Clinical and field evaluations

5.5.1 Socio-demographics and household set-up

At the enrolment and later visits where necessary, a series of questions will be asked of each individual participant in relation to their socio-demographic information including their date of birth, sex, tribal group, education and occupation. Any relevant medical information that is identifiable from ante-natal cards (ANC), or infant welfare cards (IWC) (especially for younger children) will be recorded such as birthweight, previous medical diagnoses and allergies.

For each household data will be collected relating to the household set-up including the number of buildings, number of rooms, accessibility for non-household members, sleeping arrangements, mosquito net use/coverage, household income, water access and proximity to community meeting points.

At subsequent monthly visits, individuals will be asked to briefly update some of their sociodemographic details such as occupation, school attendance and any other factors that may change throughout the year, and to complete any missing data. Similarly, alterations to household set-up will also be collected.

5.5.2 Social-mixing behaviour

For all participants at enrolment and monthly visits, and in more detail for the incidence cohort at each monthly visit, questions will be asked of individuals regarding their social-mixing behaviour. These questions will attempt to capture details about individuals' frequently visited places outside the household. They will be asked which places they visit, such as school, a workplace, shops, markets, places of worship, and how frequently they visit them. The location and proximity of these places will be mapped using GPS data by the field team. For the more detailed extended social-mixing behaviour collected in the intensified swabbing cohort, we will collect data on places visited in the form of a daily diary, listing with as much detail as possible the locations visited, people spent time with in close-proximity and events attended.

5.5.3 Medical and drug history

At enrolment, a focused past medical history will be taken from each individual including any regular medication taken, vaccination history, previous diagnoses and previous history of skin or throat infections specifically. Also at enrolment and at each subsequent visit for all cohorts, a brief history of recent medication (particularly antibiotics) and current clinical symptoms will be taken, including details of any recent healthcare setting attendance including traditional healers.

At unscheduled visits, a clinical history of the presenting complaint, medication usage and healthcare attendance will be taken to capture information related to any potential GAS infections, but also to inform immediate and subsequent medical management of other complaints.

5.5.4 Clinical examination and vital signs

At the enrolment visit, all participants will undergo a physical examination including vital signs to provide a baseline. Vital signs collected will include axillary temperature, pulse rate and respiratory rate. Adults (over 18 years) will also have blood pressure recorded.

Participants will then undergo a physical examination which will include an examination of the pharynx and associated lymph nodes, and a full body examination of the participant's skin, to identify any pyoderma lesions, and other relevant skin conditions. Care will be taken to perform the full body examination with appropriate privacy and verbal consent obtained at the time. Portable screens will be used to maintain privacy when necessary. Participants' genitals will only be examined if they specifically report (or the parent reports, in the case of children) the presence of a lesion and verbally consent for the study nurse examine them.

At later scheduled visits, monthly visits for the main cohort, and weekly visits for the more intensive cohorts, participants will undergo the physical examination including throat and skin, but will not have vitals and anthropometry recorded unless they are reporting symptoms suggestive of a GAS infection. If they are symptomatic, a clinical history will be taken and fuller clinical examination of the presenting complaint will be done, in addition to recording vital signs.

At unscheduled visits participants will also have their vitals recorded in the same way, and a clinical history and focused clinical examination will be done.

5.5.5 Clinical samples

At each visit, participants will have clinical samples collected according to the sampling schedule outlined in table 1.

5.5.5.1 Oropharyngeal swab

Oropharyngeal swabs will be collected from each participant using standard techniques. After sample collection, the swab be aseptically placed in liquid Amies (or similar) transport solution and maintained at 2-8°C in a cold box until processing in the laboratory. Study nurses collecting the sample will take care to use appropriate personal protective equipment (PPE). Detailed procedures for swab collection and transport will be outlined in the relevant study specific procedure.

Oropharyngeal swabs will be collected in exactly the same way for participants complaining of symptoms that could be consistent with acute pharyngitis at unscheduled visits

5.5.5.2 Normal skin swab

Normal skin swabs will be collected with the intention of identifying any GAS present on the skin, rather than differentiating skin site. It is not clear which skin sites are most likely to be colonized by GAS, therefore in order to maximise sensitivity, multiple skin sites will be swabbed with the same swab.

Swabs will be obtained using modification of a standard skin microbiota swabbing technique (24-27) in which the swab head is soaked in sterile saline solution prior to skin swabbing. The swab will be taken from 2cm by 2cm squares of skin on the forehead, both forearms both lower legs, and then placed aseptically in liquid transport medium and stored at 2-8°C in a cold box until processing in the laboratory.

5.5.5.3 Pyoderma wound swab

Pyoderma skin swabs will be taken in the field from any participants with evidence of pyoderma. Pus will be expressed if necessary. Skin swabs will be placed in liquid transport medium until they reach the lab. They will be refrigerated at approximately 2-8°C in cool boxes until arrival at the laboratory.

5.5.5.4 Dried blood spot

Dried blood spot (DBS) samples will be collected onto dried blood spot collection cards from a finger prick on the participant. The finger will be cleansed with alcohol and allowed to dry before the finger prick is made. The DBS collection card will be left to dry at room temperature overnight before processing. Storage and transportation will be at room temperature.

5.5.5.5 Blood sampling

The study team will be trained to perform venepuncture on site. In the case that the head of the household, all participants aged over 18, and all guardians of children under 18 verbally consent to venepuncture for blood to be taken on site, this will be performed within the household. An alternative option will be given to every household, for an appointment to be made at a specified time to attend Sukuta Health Centre, where venepuncture for blood serum will be performed by members of the study team.

Blood will be taken from participants using peripheral venepuncture by a trained nurse or doctor from the study team. Blood samples will be collected in serum separation tubes using aseptic technique, ensuring appropriate PPE is used. Training will be provided based on the specifics outlined in the study specific protocol governing this process.

Blood serum samples will not be obtained from participants under the age of 2 years.

5.5.5.6 Salivary samples

Salivary samples will be collected using an oracol swab from participants at the time points specified in table 1. Training will be provided on sample collection for the study team based on the specifics outlined in the study specific protocol governing this process. Once obtained, the swab will be immediately placed in the collection tube according to the manufacturer's instructions.

5.5.5.7 Additional clinical samples

Any additional blood or other samples (e.g. urine) required for the routine clinical assessment and care of a participant may be obtained in the field or at MRCG clinical services as indicated with the verbal consent of the participant or parent/guardian of participants under 18 years.

5.5.5.8 Environmental swabbing

At monthly household visits, environmental swabs will be taken from common touch points in the household. At the enrolment visit, an assessment will be made by the study team lead to identify 2 surfaces to swab within the household which are commonly touched by multiple people and are rarely cleaned. This decision will be made collaboratively with the study team taking advice from the household members. Such potential swabbing points might include door handles, table surfaces, curtains, benches, chair handles etc. Once 2 surfaces have been decided for the household, those will be the 2 surfaces swabbed at each subsequent visits.

Environmental swab collection technique will be described in detail in the relevant study specific protocol governing the process. One swab will be used for each swabbing point. The swab tip will be soaked in sterile saline solution prior to swabbing, and will be rubbed slowly and thoroughly over the surface (up to 50cm) 3 times reversing direction between strokes. Once collected, the swab will be aseptically places in liquid transport medium, and stored at 2-8°C in a cold box until processing in the laboratory.

For the personal and household hygiene visit, additional environmental swabs will be collected from a wider range of common touch points in the house, and a swab will be soaked in water taken from the household greywater source.

5.5.5.9 Settle plates

A settle plate will be used at each monthly household visit to passively capture the presence of airbourne GAS within households. A culture petri dish pre-prepared with blood agar will be placed at a suitable point in the main social indoor room of the household e.g. the parlour, sitting room, or main shared bedroom. Ideal placement of the settle plate would be at least 1 metre off the floor, 1 metre away from the walls and other large obstacles. The plate will be left for 1 hour, then retrieved and stored at 2-8°C in a cold box until processing in the laboratory.

5.6 Laboratory evaluations

5.6.1 Sample transport

All swabs and clinical samples taken in the field, with the exception of DBS collection cards, will be stored as soon as possible in a cold box maintained at 2-8°C and transported to the MRCG Fajara laboratories for processing the same day.

5.6.2 Culture procedures

Oropharyngeal, pyoderma, normal skin and environmental swabs will be processed in the same way. Swabs will arrive at the laboratory in 1ml of liquid transport medium (STGG, Amies or similar). On arrival at the lab, after ensuring that the swab is inside and the lid is properly closed, the swab will be briefly vortexed in the transport medium. After vortexing, the swab will be removed and streaked onto a blood agar culture plate, and then discarded. The remaining transport medium will be stored at -70°C for subsequent use if reculturing is required, and for PCR analysis. Prior to storage the transport

medium may be separated into various aliquots. The detailed procedure will be outlined in the relevant study specific protocol.

The culture plates will be incubated overnight at 37°C and assessed for the presence of beta-haemolytic colonies. Colonies with clear beta-haemolysis will be picked and replated for purity overnight at 37°C. The following day, the colonies will be identified using catalase testing, and if negative then latex agglutination testing for Lancefield group A, C and G. Catalse positive colonies will be tested for *Staphylococcus aureus* using latex testing.

Any group A, C or G streptococci isolates identified will then be stored in glycerol broth at -70°C for later revival, DNA extraction and whole genome sequencing (WGS). *S. aureus* colonies will also be stored for later analysis.

Antimicrobial susceptibility testing by disc diffusion using standard CLSI procedures will also be performed on group A, C or G streptococcal isolates identified.

5.6.3 Quantitative PCR

Reserved transport medium from some samples will undergo quantitative PCR testing using GAS-specific (*spy*-gene or other GAS-specific target) real-time PCR. Stored aliquots will undergo DNA extraction followed by quantitative PCR in order to obtain a measure of GAS bacterial load. The detailed procedures for the PCR will be outlined in the relevant study specific protocol.

5.6.4 Whole genome sequencing (WGS)

Isolates will be revived, and DNA extracted using established methods at MRCG. Library preparations and WGS (illumina Miseq) will be undertaken at the MRCG genomics facility. Quality control (fastqc), *de novo* genome assembly (SPAdes) and core genome determination (roary) will be done, followed by basic phylogenetic reconstruction using maximum likelihood (RAxML). A state-of-the-art tool (phyloscanner (28)) will be utilised to determine genotypically-linked isolates by analysing genetic diversity and relationships within and between cases simultaneously.

5.6.5 Dried blood spot (DBS) sampling

Whole blood will be collected from finger or heel prick in the field. Six drops will be collected onto DBS collection cards. Collection cards will be dried at room temperature overnight, they can then be stored at room temperature or refridgerated. Upon processing in the laboratory, 6mm punches of dried blood filter paper will be obtained, which will be eluted using a buffer solution. The resulting eluate will then undergo serological analysis for anti-GAS antigens, including streptolysin O, SpyCEP, SpyAD, GAC, and M protein.

5.6.6 Serum blood

Serum blood samples will be used to assess serological activity to GAS antigens including streptolysin O, SpyCEP, SpyAD, GAC, and M protein at baseline and at the end of the cohort. Serum blood taken at the same time-points as DBS samples will additionally contribute to validation of DBS in this setting as a reliable and reproducible assay for anti-GAS proteins. Serum samples will be stored for further immunological work including streptococcal killing assays and opsonophagocytic assays in order to explore correlates of protection from GAS asymptomatic carriage and clinical disease.

5.6.7 Salivary samples

Salivary samples will be used to assess for mucosal antibody activity to GAS antigens including streptolysin O, SpyCEP, SpyAD, GAC, and M protein. Samples will be stored for further immunological work.

5.7 Modelling

By utilising the novel data in this project, it will allow estimation of relative contributions of between and within household transmission, and transmission between symptomatic and asymptomatic individuals. A mathematical model of GAS household transmission will be designed based on the data from this study. The model will be calibrated using a combination of clinical and behavioural data, and use phylogenetic relatedness of isolates to identify transmission events between study participants. The household model will also be valuable in evaluating potential intervention strategies for future implementation within LMICs.

6 Safety and treatment considerations

As this study is observational and does not involve an investigational product, adverse events will not be formally recorded and there is no data safety monitoring board. However, while participants are enrolled in the study, the study team is responsible for certain aspects of their medical care, in particular with respect to GAS infections.

6.1 Diagnosis and management of GAS infections

6.1.1 Definition of GAS-related disease

6.1.1.1 GAS pharyngitis

Acute pharyngitis (including tonsilitis) is usually defined as inflammation of the pharynx or tonsils with an acute onset. GAS is only one potential cause of acute pharyngitis, and viral infection is the most common cause. Of bacterial infections, GAS infection is the most common, but others include *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Bordetella pertussis*. There are additionally a small number of fungal and non-infectious causes.

For the purpose of this study it is necessary to distinguish between:

1. **Asymptomatic colonisation** of the pharynx with GAS and **symptomatic infection** of the pharynx by GAS

   and

2. **Symptomatic infection** of the pharynx **by GAS** and **symptomatic inflammation** of the pharynx **by another infectious or non-infectious cause**

Therefore, we will use the following definitions:

Asymptomatic pharyngeal GAS colonisation

GAS identified on culture from a swab taken from the oropharynx of a participant who at the time of sampling did not complain of any of the following symptoms: *fever*, *sore throat*, *difficulty swallowing*, *cough*, or *hoarse voice*, and on clinical examination did not have any of the following features: *inflammation or erythema of the pharynx, tonsils, palate or epiglottis, pus or exudate on the tonsils, swelling of the cervical or submandibular lymph nodes, a scarlatiniform rash, or petechiae on the palate*.

Acute GAS pharyngitis
GAS identified on culture from a swab taken from the oropharynx of a participant who at the time of sampling did complain of any of the following symptoms: *fever*, *sore throat*, *difficulty swallowing*, *cough*, or *hoarse voice*, or on clinical examination did have any of the following features: *inflammation or erythema of the pharynx, tonsils, palate or epiglottis, pus or exudate on the tonsils, swelling of the cervical or submandibular lymph nodes, a scarlatiniform rash, or petechiae on the palate*.

Acute pharyngitis from a non-GAS cause
GAS not identified on culture from a swab taken from the oropharynx of a participant who at the time of sampling did complain of any of the following symptoms: *fever*, *sore throat*, *difficulty swallowing*, *cough*, or *hoarse voice*, or on clinical examination did have any of the following features: *inflammation or erythema of the pharynx, tonsils, palate or epiglottis, pus or exudate on the tonsils, swelling of the cervical or submandibular lymph nodes, a scarlatiniform rash, or petechiae on the palate*.

6.1.1.2 GAS pyoderma

Pyoderma is a an umbrella term for any form of pyogenic (pus-forming) skin infection including impetigo, ecthyma, carbuncle, folliculitis and tropical ulcer. Similarly to pharyngitis there are many infectious and non-infectious causes of pyoderma, but the most common causes are infection with *Staphylococcus aureus* and GAS.

For the purpose of this study it is necessary to distinguish between:

1. **Asymptomatic colonisation** of the skin with GAS and **symptomatic infection** of the skin by GAS

   and

2. **Symptomatic infection** of the skin **by GAS** and **symptomatic inflammation** of the skin **by another infectious or non-infectious cause**

However, with the case of normal skin swabs compared to pyoderma, the sampling site plays a significant role in the diagnosis. Therefore, we will use the following definitions:

Asymptomatic skin GAS colonisation
GAS identified on culture from a swab taken from the normal skin of a participant who at the time of sampling at the sampling site did not have the following features: *pain or itching and pus or crusts with inflammation or erythema*.

GAS pyoderma
GAS identified on culture from a swab taken from a pyoderma-like lesion of a participant which did have the following features: *pain or itching and pus or crusts with inflammation or erythema*.

Pyoderma from a non-GAS cause

GAS not identified on culture from a swab taken from a pyoderma-like lesion of a participant which did have the following features: *pain or itching and pus or crusts with inflammation or erythema.*

6.1.1.3 Invasive GAS disease

Due to the procedures put in place by this study to identify superficial GAS infections (as described in section 5.3.4) and to treat them (as described in section 6.1.3 below), it is unlikely that a GAS infection should progress to an invasive infection. However, it is possible that a participant reports to the study team or is made aware to the study team by another means who is suffering from symptoms consistent with an invasive GAS infection. Invasive GAS infection is usually associated with particularly virulent strains, and in HIC is more common in those with immunosuppression, diabetes and intravenous drug users. Invasive GAS infection usually refers to infections in which patients are systemically unwell, and GAS is identified in a normally sterile area of the body such as blood (GAS septicaemia), lungs (GAS pneumonia), deep muscle or fat tissue (GAS necrotising faciitis), or cerebrospinal fluid (GAS meningitis). A particular clinical syndrome can occur with GAS called Streptococcal toxic shock syndrome (STSS) resulting from bacterial production of exotoxins and virulence factors resulting in a cytokine cascade and the sudden onset of shock, organ failure and frequently death.

For the purposes of this study the following definition will be used:

Invasive GAS infection
GAS isolated from a normally sterile body site, including but not limited to: blood, deep muscle or fat tissue, CSF, joint fluid or pleural effusion, in a participant exhibiting any symptoms of a systemic inflammatory response syndrome or other symptoms compatible with a systemic infection.

6.1.1.4 Acute rheumatic fever (ARF)

Acute rheumatic fever is an inflammatory reaction that occurs in response to a GAS infection, classically understood to develop 2-4 weeks following an episode of GAS pharyngitis, typically in children aged 5-14 years. If left untreated, GAS pharyngitis is more likely to trigger an episode of ARF. As we will treat cases of GAS pharyngitis with antibiotics, it is less likely that episodes of ARF will occur in our cohort.

The Revised Jones Criteria are used as an international standard for diagnosing ARF, and will be used in this study. The criteria can been seen in the appendix. It may be possible within the context of this study to identify the preceding GAS infection, but this is not necessary for the diagnosis of ARF. Although not included in the criteria, a GAS positive pharyngeal swab, and raised streptococcal antibody titre can support the diagnosis.

For the purposes of this study the following definition will be used:

Acute Rheumatic Fever
A participant presenting with 2 major manifestations, or 1 major and 2 minor manifestations of ARF according to the Revised Jones Criteria (see appendix).

6.1.2 Procedure for assessing participants for GAS infection

All enrolled participants will be provided with an on-call number to call at any time when they, or another enrolled household member, is experiencing symptoms that could be compatible with any of the above GAS infections outlined in section 6.1.1. The on-call number will be staffed by a study

nurse 24 hrs a day for the duration of the study, according to an on-call rota. The study nurse taking the call will complete a rapid assessment proforma over the phone, and based on the findings, and in discussion with the on-call study physician if necessary, will make an assessment about the required timeliness for the participant to be assessed in person.

As described in section 5.3.4, the result of the rapid assessment could include an appointment to be seen the next working day, an urgent appointment the same day, evening or night, or an urgent assessment at the MRCG clinical services department.

At any urgent, or non-urgent assessment, the required study samples and CRFs will be completed only when they can safely be done so by not delaying necessary medical treatments or investigations. If in the course of the assessment, it becomes clear to the assessing nurse or physician that the diagnosis is not, or is unlikely to be due to GAS infection, they will continue to assess and manage the patient to the highest available standard of care according to WHO or local guidelines as appropriate, and will then refer on the participant to the appropriate healthcare setting for ongoing care and follow-up.

6.1.3 Management and treatment of GAS infections

The proof of presence of GAS infections requires the culture results to be completed, which may have a delay of several days, depending on culture turnaround time. Therefore it will be necessary to treat GAS infections empirically in the first instance awaiting the culture result.

For acute pharyngitis, treatment will be provided according to WHO guidelines or local guidelines as appropriate.

Pyoderma cases will be treated empirically with cloxacillin or an appropriate alternative according to best local practice and availability, as although they may not be due to GAS, another bacterial cause warrants antibiotic treatment nonetheless.

Any participant presenting with symptoms suggestive of systemic infection and possible invasive disease will be urgently referred to MRCG clinical services department (CSD) where they will be treated by CSD staff according to MRCG guidelines and best practice.

6.2 Management of other medical problems

In the course of the scheduled study visits, and the participant self-referrals for unscheduled visits, it is likely that participants will present with conditions unrelated to GAS infection, but nonetheless require appropriate management. Participants presenting with medical conditions of concern will be treated or referred as appropriate.

6.2.1 Other study related diagnoses

Other diagnoses that will be captured in the study that may be picked up during the examination include scabies, fungal skin infections, and insect bites.

6.2.2 Non-study related diagnoses

All other non-study related diagnoses will be managed or referred on by the study nurses as appropriate according to best local practice. In particular however, unnecessary antibiotic treatment will be avoided, and where there is doubt, the on-call study physician will be consulted. Any fever will be investigated with a malaria RDT in the first instance, and any participant presenting with symptoms meeting the criteria for COVID-19 testing will be referred to the MRCG CSD for appropriate investigation, isolation and management.

7 Discontinuation criteria

7.1 Participant's premature termination

Any participant may withdraw their consent to participate in the study at any point in their enrolment without giving a reason why. If a participants does withdraw their consent, they will be asked whether any data or samples already collected can be used in the analysis or not. An individual household member withdrawing their consent and terminating their involvement will not require the entire household to be withdrawn from the study, but if a majority, or a substantial number of individuals from the same household withdraw, a decision will be made by the PI, study team and co-investigators as to whether the household should be withdrawn.

7.2 Study discontinuation

In the event that the study can no longer continue safely due to any reason, such as MRCG limiting research activities or The Gambia Government introducing new public health restrictions which make the study unable to continue, the study will be discontinued following a decision by the PI, study team and co-investigators.

8 Statistical considerations

The primary outcome measures used to determine sample size were:

1. GAS carriage prevalence, and
2. GAS carriage incidence over 12 months.

In HIC, GAS pharyngeal carriage prevalence in children is 2-17%(12, 13), and in Uganda is 15.9% (29). Our study also includes adults, in whom carriage is lower, but will use pooled skin and pharyngeal carriage as our outcome measure, which will likely increase prevalence in turn. **We therefore estimate a pooled prevalence of 15**%.

GAS pharyngeal carriage yearly incidence in children in the US was shown to be 27-32% (12). We found a skin infection incidence of 592/1000 child years in The Gambia during an influenza vaccine study follow-up (unpublished data) and of which ~50% are likely due to GAS (30). As we are including adults with a likely lower incidence, **we estimate a yearly incidence of 20%.**

The sample size was calculated for the primary objective, GAS carriage prevalence, using the formula below to measure the estimated prevalence of 15% with a precision of ±5%.

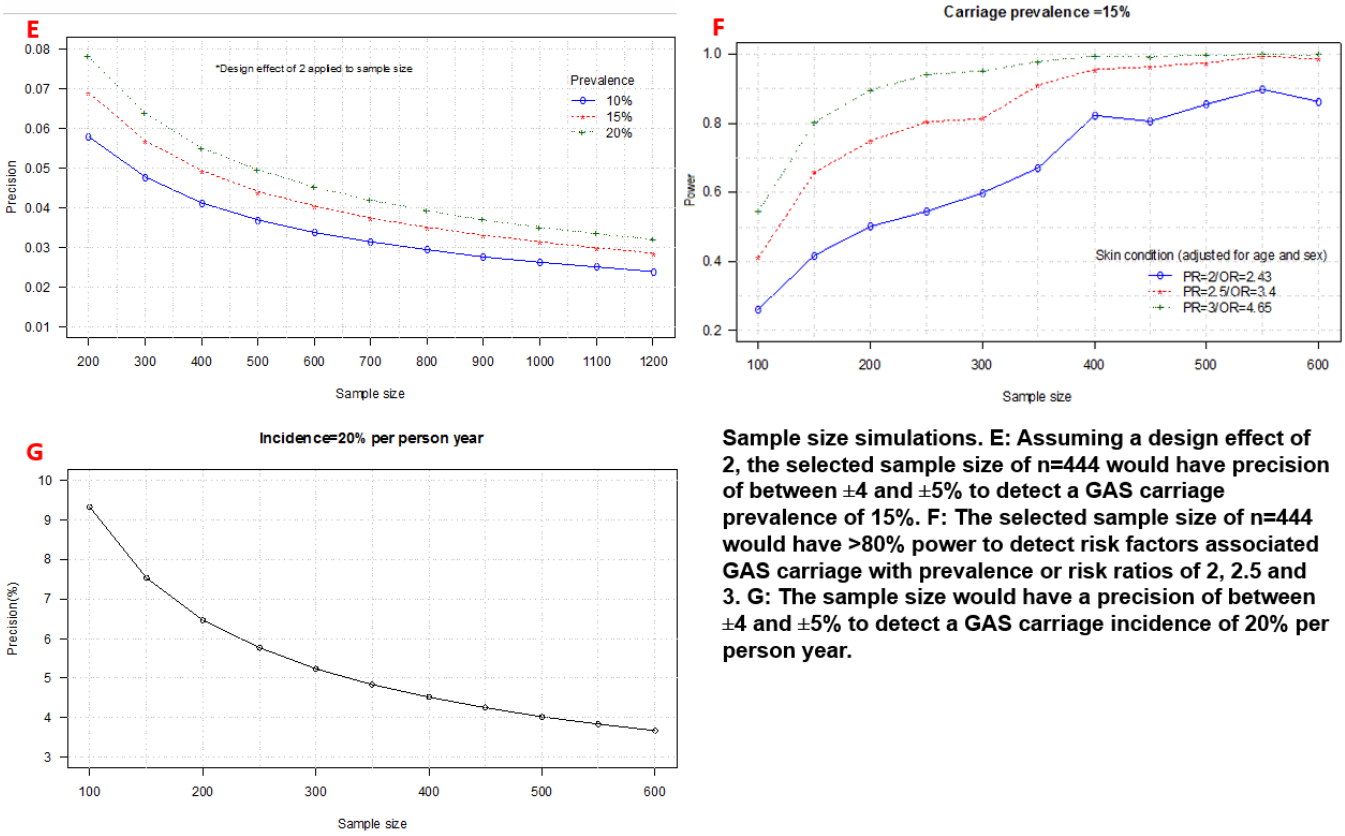
$$n = \frac{{Z_{\alpha/2}}^2 \times p \times (1-p)}{e^2}$$

Where *p* is predicted proportion and *e* is desired precision.

Using *Z*=1.96 for *α*=0.05, *p*=0.15 and *e*=0.05 we require a sample of 196. Intracluster correlation is unknown, **therefore we used a conservative design effect of 2**, which allowing for 10% drop-out rate gives a required sample size of 431.

We therefore propose to recruit 45 households, which with an average household size of 10, will equal approximately 450 individuals for the main cohort.

This sample size would provide adequate power for precise estimates of prevalence and incidence of GAS carriage and to detect risk factors for GAS carriage (Figures E-G).



Sample size simulations. E: Assuming a design effect of 2, the selected sample size of n=444 would have precision of between ±4 and ±5% to detect a GAS carriage prevalence of 15%. F: The selected sample size of n=444 would have >80% power to detect risk factors associated GAS carriage with prevalence or risk ratios of 2, 2.5 and 3. G: The sample size would have a precision of between ±4 and ±5% to detect a GAS carriage incidence of 20% per person year.

9 Data handling and record keeping

9.1 Unique study ID numbers

Each household on the GPS location list will be given a household ID number (HID) which will be three digits long (e.g. 001, 002). This number will not change regardless of whether the household provides consent and is enrolled or not. The HID will be recorded on the sensitization form and sensitization log book when the household undergoes sensitization. If a household reaches the stage of informed consent, each ICD will be documented with the HID number at the top. Each household member who then consents will be given, at that point, a two digit individual study number (e.g. 01, 02, 03), according to the number of household members and the order in which they are consented. The two digit individual study number will be recorded on that individual's ICD, in addition to the HID.

All consented individuals will then have a unique participant ID number (PID) consisting of the three HID digits (e.g. 004), followed by their two digit individual study number (e.g. 02), and then a check digit calculated by the Damm algorithm (e.g. F). Their PID number will therefore consist of 5 numbers and one letter (e.g. 00402F). This number is unique to each participant in the study, and will be

recorded on every CRF and other study document completed by the study team. After sensitization and consent, no participant identifiable data will be recorded, and participants will only be identified by their PID.

9.2 Data management and processing

Field data will be collected on electronic case report forms (eCRF) inputted on tablet computers by the field team. The questionnaires will be designed using REDCap™ electronic data capture software hosted at MRCG. Data will be collected offline and synced with the secure database at the end of each day. Data generated in the laboratory will be inputted onto the same database. Written informed consent will be sought from all participants prior to any study activities and before any data is collected.

Questionnaires will be designed with up-front data quality checks including reference ranges and dropdown menus to minimise incorrect data entry. Additionally, after completion of the study, a data checking process will be performed running queries to check for incomplete or nonsense data.
All data will be kept confidentially, and electronic data encrypted. Each participant will be assigned a unique study ID, so that no person identifiable data will be kept on the database. Any person identifiable data will be held securely and will not be available to anyone other than those in the investigator team.

9.3 Data sharing and access

Data will be handled in accordance with the data management SOPs of MRCG which is fully compliant with GDPR regulations. All documents used will be version controlled and dated. Anonymised data will be held in the study database for a minimum of 10 years following project completion, in compliance with LSHTM's Records Retention and Disposal Schedule. Anonymised raw study data and analysis code will be deposited in the LSHTM Data Compass repository on publication of study outputs, and will be available upon request for scientific purposes.

Whole genome sequencing data will be generated using the illumina Miseq platform at MRCG. Raw sequence data will be in the form of fastq files and initially stored on the high performance cluster (HPC) at MRCG. Data management and analysis will be performed on pipelines established on both the MRCG HPC, as well as cloud-based servers such as the MRC CLIMB platform (used currently by Dr. de Silva's group and others at MRCG). Raw sequence data will be archived at MRCG according to their data archiving procedures. Certain processed data fields from genomic analysis (e.g. *emm* type) will be included as variables in the study REDCap™ database. Sequence data along with links to relevant metadata will be submitted to a public sequence repository (e.g. genbank) as is standard practice, on publication of study outputs. Analysis pipeline and code will be made openly available via GitHub on publication of the study.

9.4 Source documents and access to source data

The Principal Investigator will maintain appropriate medical and research records for this study in compliance with the principles of good clinical practice and regulatory and institutional requirements for the protection of confidentiality of participants. The study team members will have access to records.

The authorised representatives of the sponsor, the ethics committee(s) or regulatory bodies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) for the participants in this study. The clinical study site will permit access to such records.

10 Ethical considerations

This study is conducted in accordance with the principles set forth in the ICH Harmonised Tripartite Guideline for Good Clinical Practice and the Declaration of Helsinki in its current version (see appendix), whichever affords the greater protection to the participants.

Ethical approval for the study will be sought from the MRC Scientific Coordinating Committee and the joint MRC/Gambia Government Research Ethics Committee. As described above, RHD carries a significant economic and health burden in The Gambia, as do superficial GAS infections. The results of the study will contribute valuable information to the global scientific community, as well as to policy makers in The Gambia. By raising awareness of the burden of these common infections and the importance of reducing GAS carriage and transmission, the study aims to provide a basis for future work which will focus on reduction strategies.

10.1 General considerations on human subject protection

10.1.1 Rationale for participant selection

Since GAS carriage and infection can be present in anyone, regardless of age or sex, we chose not to exclude anyone on these bases. Sukuta is the site of recruitment as this builds on our previous data from this area. Selection of households for participation will be done by random geographical selection. A set of random GPS coordinated within Sukuta will be generated, and the study team will approach the nearest household to that location for participation. For the purposes of enrolment in the study, a household will be defined according to The Gambia Demographic and Health Survey 2013 definition: “a household [is] defined as a person or a group of related or unrelated persons who live together in the same dwelling unit(s) or in connected premises, who acknowledge one adult member as the head of the household, and who have common arrangements for cooking and eating.”

We made the decision to exclude households with fewer than 3 members and those without any children, as smaller households would make household transmission analysis less relevant and may not provide information about within household transmission that we require.

10.1.2 Evaluation of risks and benefits

Risks:
There is a small risk of discomfort to individuals during the sampling procedures. Taking of oropharyngeal and pyoderma wounds swabs may be slightly uncomfortable, but is safe and brief. Salivary samples are collected using foam oracle swabs which do not hurt at all. Dried blood spot collection may be uncomfortable due to a pinprick with a lancet, and blood serum collection may cause mild discomfort and a slight bruise.

Apart from sampling discomfort, some of the questions asked may feel slightly intrusive to the participants, including asking about social mixing behaviour, however it will always be made clear to participants that they can choose not to answer any question.

Benefits:
The benefit to the participants and their household is that they will have regular visits from nurses examining them for evidence of GAS infections and other medical problems. Any GAS infections and some other common medical problems will be provided with treatment at the household visits. They will therefore receive regular medical check-ups over the course of enrolment in the study, likely resulting in prompt diagnosis of any medical problems.

Furthermore, participants will have access to the doctors and nurses of the study on-call team in case of any medical problem that may arise, who can advise them what to do, provide prescriptions or referrals as appropriate to ensure that participants receive the best level of local care available.

The community and country additionally stand to benefit from the findings of the study in that they will inform future GAS control strategies in countries such as The Gambia, and the study will place the community in an ideal position to be considered as a site for potential future GAS vaccine trials.

10.2 Informed consent

Written informed consent will be obtained before any study activities are undertaken. The informed consent document (ICD) will be read line-by-line to the participant or participant's parent/guardian in the language of their choice. If they agree to participate, they will be asked to sign (or thumbprint) the consent form. If the parent has more than one eligible child, they will be asked to sign a consent form for each child. The option to consent for the future use of samples on study completion will also be given on the same page but the provision of such additional consent is not required for participation in the main trial. The ICD will make clear the purpose of the study, include information about the use of the samples, and describe the extent of the examination required. Participants will be free to withdraw from the study at any time without giving a reason

10.3 Participant confidentiality

Participants' identifiable data such as names, and telephone numbers will be kept in separate log books and utility databases separate from the overall study database. Access to these logs and databases will be controlled, ensuring that only the necessary persons have access to participants personal details. All study data collected will be anonymised and linked to the individual's unique PID number (see section 9.1).

10.4 Future use of stored specimen

The informed consent documents will contain an option for participants to consent for the future use of samples collected in the study for additional research, and it will be made clear to participants during their informed consent process that their choice in this matter does not affect their involvement in any other way.

11 Financing and insurance

The study will be funded by the PI's clinical PhD fellowship award through the Wellcome Trust via LSHTM. Additional funding will be sought for additional analyses of samples, and other study activities which may be possible following submission of protocol amendments, where possible from other funding sources.

12 Publication policy

During the study design and protocol preparation we will maintain contact with a number of collaborators in the GAS field around the world, and at MRCG. This will allow us to refine the research design and endpoints according to advice and new evidence that might emerge before the start of the study.

The study results will be published promptly in peer-reviewed journals and promoted through the MRC communications department and strategy and through social media where appropriate. Data will be submitted as abstracts to be presented at international conferences such as the European Congress of Clinical Microbiology and Infectious Diseases and the Lancefield International Meeting on Streptococci and Streptococcal Disease.

The PI I will be the corresponding author on all published articles and will be available to discuss the results and data.

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Supplements, appendices and other documents

Appendix: Revised Jones Criteria (from CDC)

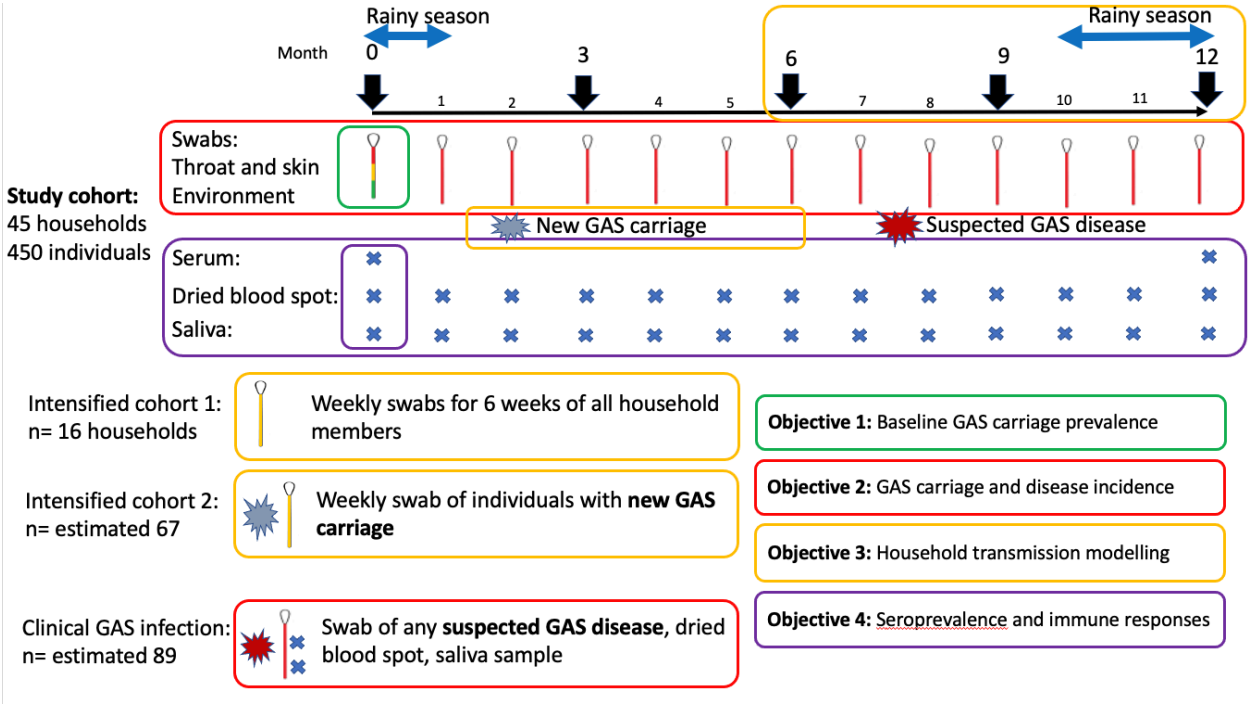
Table: Revised Jones Criteria for diagnosing acute rheumatic fever

Part A. For all patient populations with evidence of preceding group A strep infection		
Diagnosis: Initial ARF	2 major manifestations *or* 1 major plus 2 minor manifestations	
Diagnosis: Recurrent ARF	2 major manifestations *or* 1 major plus 2 minor manifestations *or* 3 minor manifestations	
	Low-risk populations*	**Moderate- and high-risk populations***
Part B. Major manifestations	Carditis • Clinical and/or subclinical Arthritis • Polyarthritis only Chorea Erythema marginatum Subcutaneous nodules	Carditis • Clinical and/or subclinical Arthritis • Monoarthritis or polyarthritis • Polyarthralgia (if other causes have been excluded) Chorea Erythema marginatum Subcutaneous nodules
Part C. Minor manifestations	Polyarthralgia Fever (≥38.5°C) Elevated acute phase reactants (ESR ≥60 mm in the first hour and/or CRP ≥3.0 mg/dl) Prolonged PR interval on electrocardiography, after accounting for age variability (unless carditis is a major criterion)	Monoarthralgia Fever (≥38°C) Elevated acute phase reactants (ESR ≥30 mm/hr and/or CRP >3.0 mg/dl) Prolonged PR interval on electrocardiography, after accounting for age variability (unless carditis is a major criterion)

ARF = acute rheumatic fever; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; mm = millimetres; mg/dl = milligrams per decilitre

*Low-risk population is defined as an acute rheumatic fever incidence of ≤2 per 100,000 school-aged children or all age rheumatic heart disease prevalence of ≤1 per 1000 population per year. Those not included in the low-risk population are defined as moderate or high risk depending upon their reference population.

Schematic of Study Design:



Appendix: Schedule of events

Visit timing	Visit window	Data and samples	Main cohort	Incidence cohort (12 households)	Duration cohort (new GAS carriers)
Month 0 enrolment visit (MV0)	-	Eligibility	X	-	-
		Sociodemographics	X	-	-
		Social mixing behaviour	X	-	-
		Household setup	X	-	-
		Medical history	X	-	-
		Physical examination	X	-	-
		Oropharyngeal swab	X	-	-
		Normal skin swab	X	-	-
		Blood serum*	X	-	-
		Dried blood spot	X	-	-
		Salivary sample	X	-	-
		Environmental swabs	X	-	-
		Settle plates	X	-	-
Weekly visits	+/- 3 days	Medical history	-	X	X
		Extended social-mixing behaviour	-	X	-
		Physical examination	-	X	X
		Oropharyngeal swab	-	X	(X) *if previously positive*
		Normal skin swab	-	X	(X) *if previously positive*
Monthly visits (MV1, MV2, MV3 etc. up to MV12)	+/- 7 days	Update sociodemographics	X	X	-
		Medical history	X	X	-
		Social-mixing behaviour	X	-	-
		Extended social-mixing behaviour	-	X	-
		Update household setup	X	X	-

		Physical examination	X	X	-
		Oropharyngeal swab	X	X	-
		Normal skin swab	X	X	-
		Blood serum*	X	X	-
		Dried blood spot	X	X	-
		Salivary sample	X	X	-
		Environmental swabs	X	X	-
		Settle plates	X	X	-
Unscheduled visits (may occur at scheduled visits if symptoms present)	-	Clinical history	X	X	X
		Physical examination	X	X	X
		Wound or throat swab	(X) *if applicable*	(X) *if applicable*	(X) *if applicable*
		Salivary sample	(X) *if applicable*	(X) *if applicable*	(X) *if applicable*
		Blood sample	(X) *if applicable*	(X) *if applicable*	(X) *if applicable*
Personal hygiene visit (done at another monthly visit		Personal and household hygiene questionnaire	X		
		Extended environmental swabbing	X		

Appendix:

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964
and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available

the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.