PAEDRIATRIC SCHISTOSOMIASIS: FILLING THE GAPS

CORRESPONDENCE BETWEEN SERUM AND DRIED BLOOD SPOT RESULTS FOR QUANTITATION OF S. HAEMATOBIUM ESPECIFIC ANTIGEN IN PRESCHOOL-AGED CHILDREN (PSAC) IN ANGOLA

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ABSTRACT

Background: Urinary schistosomiasis is a disease produced by *s. haematobium* which principally produces microhaematuria, anaemia, poor nutrition and growth in children. It has shown that in schistosome-endemic areas preschool-aged children (PSAC), that is, ≤5 years, are at risk of infection. They have higher vulnerability, low infection with less diagnosis because of the low sensitivity and specificity of the gold standard. Recent research has demonstrated UCP-LF CAA as useful diagnosis test for low burden schistosome infections.

Objective: The purpose of this research is to assess the correspondence between Serum and Dried Blood Spot results for Quantitation of Schistosoma CAA on UCP-LF. The secondary objective is to assess the prevalence of schistosomiasis s. haematobium in preschool-aged children in Cubal and to validate CAA testing in DBS collected onto Whatman 903 Protein Saver cards.

Design: A diagnostic test study with a **cross-sectional** design carried out in Cubal city.

Methods: Prospective **consecutive non-probabilistic sampling** will be performed. Blood samples, DBS samples and urine samples will be recollected from children under 5 years who haven't got recent schistosomiasis treatment (within the past 6 months). We will compare the infection status (positive/negative) with UCP-LF CAA in DBS and blood against the ''gold-standard'' diagnosis of schistosomiasis, recommended by WHO. Sensitivity, specificity, predictive values will be compared analytically.

Keywords: schistosomiasis, PSAC, *s. haematobium,* UCP-LF CAA

ABBREVIATIONS

CAA: Circulating Anodic Antigen

CCA: Circulating Cathodic Antigen

DALYs: Disability-Adjusted Life-Years

MDA: mass drug administration

NDT: neglected tropical disease

NPV: negative predictive value

PC: Preventive chemotherapy

POC: point-of-care

PPV: positive predictive value

PSAC: pre-school aged children aged => 1 and < 5=>

PZQ: praziquantel

SAC: school aged children aged => 5 and <>

SCH: schistosomiasis

SSA: Sub-Saharan Africa

TCA: trichloroacetic acid

UCP-LF: up-converting phosphor lateral flow

WHO: World Health Organization

YLDs: Years Lived with Disability

INTRODUCTION

SCHISTOSOMIASIS

Schistosomiasis (bilharzia or bilharziasis) is an acute and chronic parasitic infection caused by a trematode of schistosome gender.

It is a neglected tropical disease (NTD) being prevalent in the marginalized sectors of society: those living on 1 dollar a day with limited access to safe water and adequate sanitation provision in tropical and subtropical countries. It has been reported even among the poor living in the world's leading Group of Twenty countries (1).

Two major disease-causing schistosomes in Africa, South America, and the Middle East are *Schistosoma mansoni* and *S. haematobium* (2). *Schistosoma mansoni* is found primarily across sub-Saharan Africa and some South American countries (Brazil, Venezuela, Suriname) and the Caribbean. Whereas *S. haematobium* is located in 52 countries in Africa and pockets of the Middle East (3). There are other types of schistosomiasis as s. *japonicum* affecting Asia (despite its name, it has long been eliminated from Japan), *Schistosoma mekongi* (Laos, Cambodia), and *Schistosoma intercalatum* (West and Central Africa), and species adapted to other mammals which can occasionally infect humans, like *Streptococcus bovis* and *S. magrobowe*i.

Epidemiology

Being a very common disease worldwide it is endemic in 74 countries, affecting around 240 million people every year (90% in sub-Saharan Africa). It is estimated that 436 million people are in risk of being infected (2).

After malaria, schistosome infection is second in the rank of parasite illness (3). It causes about 280,000 deaths annually only in the African region, being the most deadly NTD (4). Also, 3.3 million disability-adjusted life years (DALYs) are lost per year (5)(6).

Higher infection prevalence appears in 5 to 20 years old population, with no differences related to sex (7).

Table 1. Shows the current estimated total number of individuals with morbidity and mortality due to *S. Haematobium* infection in Sub-Saharan Africa (2)

S. haematobium	Estimated morbidity and mortality (millions)
At risk of infection	436
Infected	112
Haematuria during previous 2 weeks	71 (52-89)
Dysuria during previous 2 weeks	32 (17-55)
Minor bladder morbidity (detected by ultrasound)	76 (67-92)
Major bladder morbidity (detected by ultrasound)	24 (15-31)
Moderate hydronephrosis	9.6
Major hydronephrosis	9.6
Non-functioning kidney	[1.7]
Non-functioning kidney (deaths/year)	[0.15]
Bladder cancer (deaths/year)	
Male	[0.011]
Female	[0.0023]

Current studies demonstrate an increase of SCH prevalence due to hydric resources development projects and population movements to bigger cities with a necessity of electricity and water resources (8).

Europa is not endemic however, due to the increase of people travelling possibilities (international travels, migration, tourism…) schistosome infection diagnose is common. There was even an outcome of *S. haematobium* in Corsica, France (8).

Infection

People get in contact with schistosome infected water during daily activities. Parasite larvae are released in fresh water by snails (who have been its host since then) and penetrate the human skin. Inside human vessels larvae becomes an adult schistosome. Then they migrate to venous plexus of the bladder (*s. haematobium*) or mesenteric venules (*s. mansoni*) where the females release their eggs with the urine/stool to the exterior, following the parasite vital cycle. However, some eggs stay in tissues producing immune reaction and progressive organ damage. The whole process is represented on **Figure 1** (10).

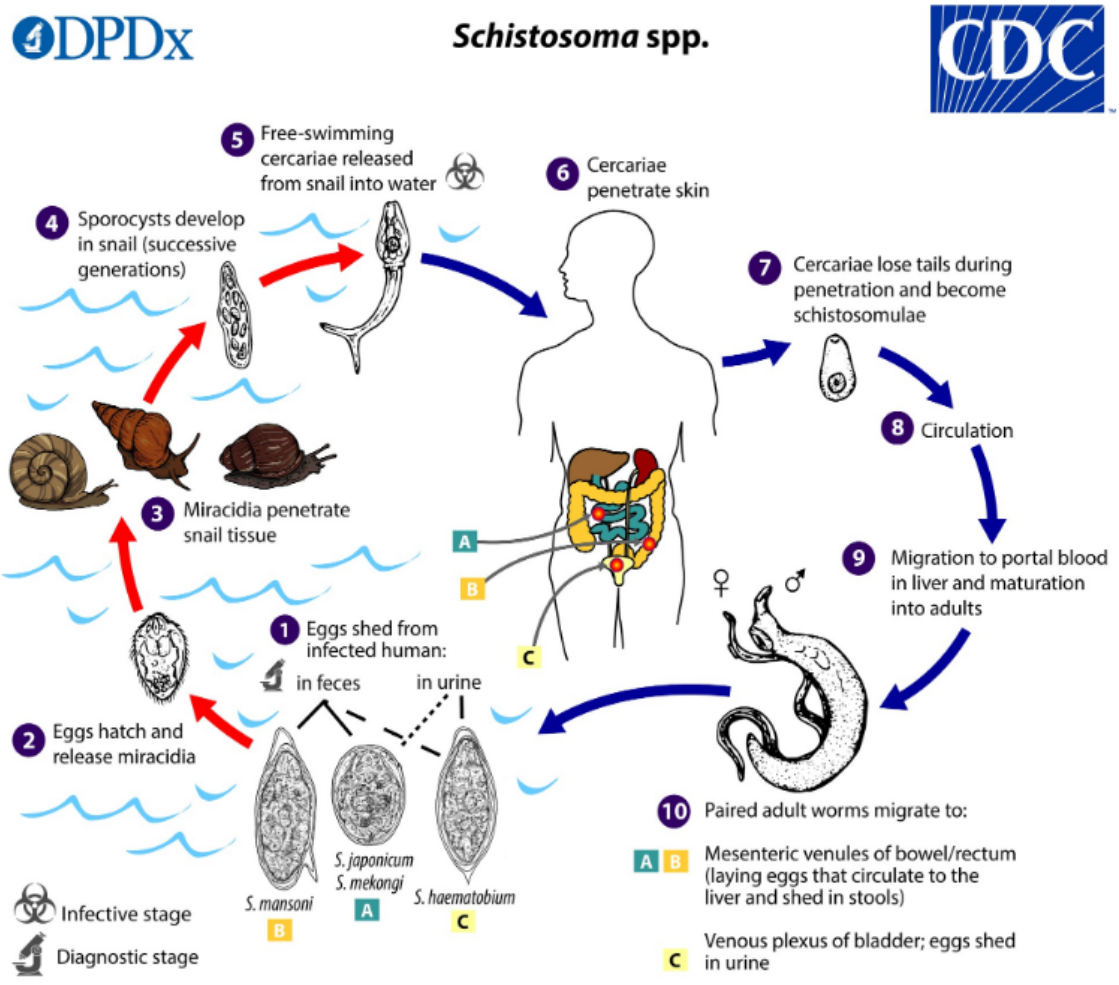

Fig. 1: *Schistosomiasis* vital cycle (9)

High-risk groups

There are three high-risk groups:

- Pregnant and lactating women while exposed to infested water during domestic activities
- Preschool and school-aged children
- Adults from special groups – (occupations involving contact with infested water, viz., fishermen, farmers, irrigation workers, or women in their domestic tasks) to entire communities living in SCH-endemic settings.

Notably, women and children (under the age of fourteen) spend most of time in water bodies in order to wash their clothes, do the dishes, fetch water and bath, thus putting them at the highest risk level (1).

Symptoms

Schistosomiasis progresses in three phases: acute, chronic, and advanced condition. The incubation period for patients with acute schistosomiasis is usually 14-84 days. However, many people are asymptomatic and have subclinical disease during both acute and chronic stages of the infection.

If there are symptoms a maculopapular eruption (rash) initially appears **Figure 2** (10) at the site of percutaneous penetration of cercariae, lasting two days. People with acute infection, also known as Katayama syndrome, may present rash (cercarial dermatitis), fever, headache, myalgia, fatigue and respiratory symptoms (non-productive cough). Often eosinophilia is present (3). The first infection in most endemic areas occurs early at pre-school age. At this age group, clinical symptoms upon schistosome exposure and infection (e.g., cercarial dermatitis and fever) may go unrecognised or be mistaken for other illnesses such as malaria, which presents similar symptoms (e.g., fever) (11).

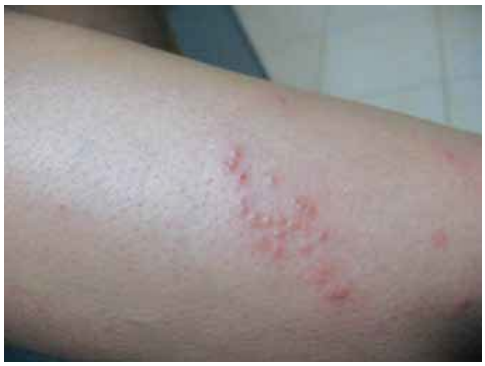

Fig 2: maculopapular eruption (10)

Nevertheless, the chronic condition exists due to: 1) lack of appropriate treatment and 2) the host's immune response to schistosome eggs deposited in the tissues and the granulomatous reaction evoked by the antigens secreted by the schistosome parasites (12).

In children, infection with the parasites causes haematuria, dysuria, nutritional deficiencies, anaemia, growth retardation, decreased physical performance and impaired memory and cognition. If left untreated, the disease can progress to bladder wall pathology, hydronephrosis (*s. haematobium*) and hepatosplenomegaly (*s. mansoni*). In adulthood, chronic schistosomiasis can also affect reproductive health, with manifestations including lesions of the cervix and vagina, vaginal bleeding and nodules in the vulva in females (increased risk of acquiring HIV-1 infection). While in males symptoms include pathology of the seminal vesicles, prostate and other genital organs (13). Also, urogenital schistosomiasis can cause renal disease and bladder cancer, being the major cause for these diseases in Africa and the Middle East (5). The schistosome infection can also produce neuroschistosomiasis, which is considered as the most severe clinical syndrome of SCH (14).

Diagnostic tools and techniques

Schistosomiasis' gold standard test is the **microscopy** detection of parasite eggs in stool or urine specimens. For urogenital schistosomiasis (*s. haematobium*), a filtration technique using nylon, paper or polycarbonate filters is the standard diagnostic technique recommended by WHO. However, the urinary excretion of eggs is not uniform

(15) and the test can be insensitive, particularly in light-intensity infections, and labour intensive (16). Microscopy sensitivity can be improved by repeated screening, but this brings up the total cost of diagnosis for each person, manpower needs and resources, which might be unaffordable. This inability to carry out repeated testing will result in an underestimation of the true burden of the infection, needing a more sensitive diagnostic and cost-effective tools. In schistosomiasis screening campaigns, **haematuria** has been found to be significantly associated with *S. haematobium* infection(17). Macro- or micro-haematuria can be assessed using dipstick or also with **questionnaires** for self-reported urinary (18).

Antibodies and/or antigens detected in blood or urine samples are also indicators of infection. Although, antibody detection is often criticised for its apparent lack of specificity and inability to distinguish active from past infection with 'False antibody-positive' results, particularly at the pre-treatment stage (19).

Antigen Based Detection Methods

These genus-specific proteoglycan antigens of the schistosome gut epithelium are released in the vomitus of worms. CCA- and CAA-based tests can be used to evaluate active worm burdens as well as the therapeutic response (20) (21).

Circulating anodic antigen (CAA) assay, UCP-LF CAA, uses luminescent quantitative up-converting phosphor (UCP) reporter particles and a rapid user-friendly lateral flow (LF) test format. Hence, UCP-LF CAA is an ultra-sensitive quantitation test that detects all Schistosoma spp. in blood, urine, or any other body fluid (22).

Compared to the traditional diagnosis of schistosomiasis by microscope, schistosome antigen testing offers advantages of single sample collection, elimination of labour-intensive work, and enhanced sensitivity. As we can see in Zanzibar studies, UCP-LF CAA assay showed significantly higher sensitivity than single urine filtration for *S. haematobium* detection (23). Antigen testing has also been recommended for serologic screening programs in which repeated examinations of urine and stool are logistically not possible and for diagnosis of young children, from whom obtaining urine sample is

difficult. In addition, the ability of CAA test to detect a single pair of worms (early stage infection), correlation of the test results (CAA levels) to egg production and worm burden makes this assay a true measure of schistosomiasis infection (22).

Applicability of this test is limited in field settings, as it involved a centrifugation step, including other resources that are needed for carrying out analysis, which will bring up the cost and the required personnel. Given that, the CAA assay has recently been developed into a dry-reagent lateral flow assay with a portable reader that can be easily transported to, and operated in, resource-limited settings.

CAA antigen's unique carbohydrate structure has no known biological equivalent. It is also heat-resistant and extremely stable, remaining detectable in tissue isolated from Egyptian mummies (24). This suggest that CAA might be easily measured in dried blood spot (DBS) samples. Dry blood spot (DBS) is an absorbent paper which allows collection and transport of blood specimens when cold chain and testing facilities are not available on site (useful in most low- and middle-income resource settings) (25). Two early studies that have explored this issue in several types of filter paper have shown that CAA was detectable but that available concentrations were low (26)(27). Also, the studies showed that haematocrit and patient nutritional status could affect CAA concentration measured in DBS. A third one, performed on 35 women in Tanzania, evaluates CAA concentration on Whatman 903 Protein Saver cards. In the study, CAA testing in DBS had a sensitivity of 76% and specificity of 79% compared to CAA testing in serum. This results prepare a proof of principle, allowing better performance of the study, increasing the sample and improving laboratory techniques (28).

Treatment

Praziquantel is the recommended treatment against all forms of schistosomiasis. It is effective, safe, and low-cost. Even though re-infection may occur after treatment, the risk of developing severe disease is diminished and even reversed when treatment is initiated and repeated in childhood (29).

It is safe in pregnant women, however as a general precaution, WHO recommendations state that all drugs should be taken after the first trimester (30). The side effects are

abdominal pain, diarrhoea, fever and headache (7). For small children, to swallow large tablets may cause choking or asphyxiation.

Prevention

The control of schistosomiasis is based on large-scale treatment of at-risk population groups (PC), access to safe water, improved sanitation, hygiene education, and snail control (15).

Groups targeted for preventive chemotherapy are: school-aged children in endemic areas; adults considered to be at risk in endemic areas, and people with occupations involving contact with infested water, such as fishermen, farmers, irrigation workers, and women whose domestic tasks bring them in contact with infested water; entire communities living in highly endemic areas; also, WHO recommends treatment of preschool aged children. Unfortunately, there is no suitable formulation of praziquantel to include them in current large-scale treatment programmes.

The frequency of treatment is determined by the prevalence of infection in school-age children. In high-transmission areas, treatment may have to be repeated every year for several years. Monitoring is essential to determine the impact of control interventions.

PAEDIATRIC SCHISTOSOMIASIS: PRE-SCHOOL AGED CHILDREN (PSAC)

Recent researches claimed that 123 million children are affected by schistosomiasis (31), and, at least, 50 million of them are PSAC (32).

It was thought that children under 5 years were unlikely to have much contact with water and were therefore unlikely to be heavily infected. From the age of about 6 years onwards, children used to learn how to swim and play in the water, and as a result they were much more at risk (30). However, it has been demonstrated that this age range (≤ 5 years) the exposure to infection is incremental, and almost all children in high transmission areas will have been exposed to schistosome cercariae by age one (33) showed in the **Figure 3** (34).

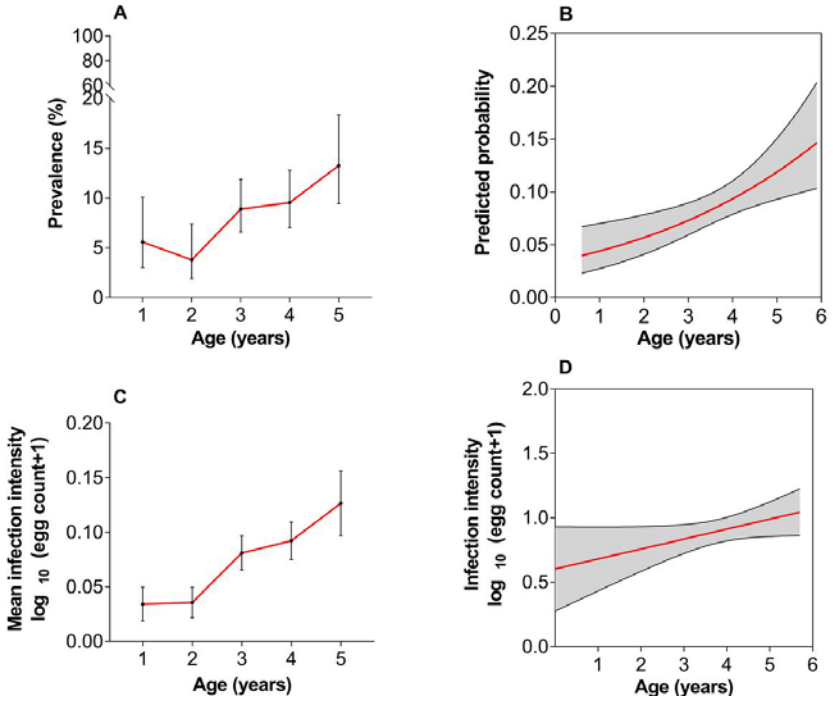

Fig 3. (A) Schistosoma haematobium infection prevalence with age; prevalence varied with age (P<0.001) and (B) age-predicted probability of infection; prevalence increased as children grew older (P=0.002). (C) S haematobium infection intensity with age; intensity varied with age (P<0.001) and (D) age-predicted intensity of infection; infection intensity increased as children grew older. Error bars indicate 95% CI (A) or SEM (C), and shaded areas indicate 95% CI; (B, D). (17)

Risk factors

PSAC are affected by several risk factors (Figure 4), including those already identified in other age group as environmental factors (temperature, seasonal rainfall patterns, and altitudes). It is been shown that the seasonal pattern of infection incidence detected is in agreement with the fact that during the dry seasons snail vectors and larval schistosomes become concentrated at permanent and slow-moving water sources, increasing the risk of infection (34).

Although for children, climate change roughness (higher temperatures, longer hotter seasons, drought…) increase recreational use of infected water resources. In addition, assive contact with infective water can increase amongst PSAC, while they are waiting for their caregivers to complete their chores (35).

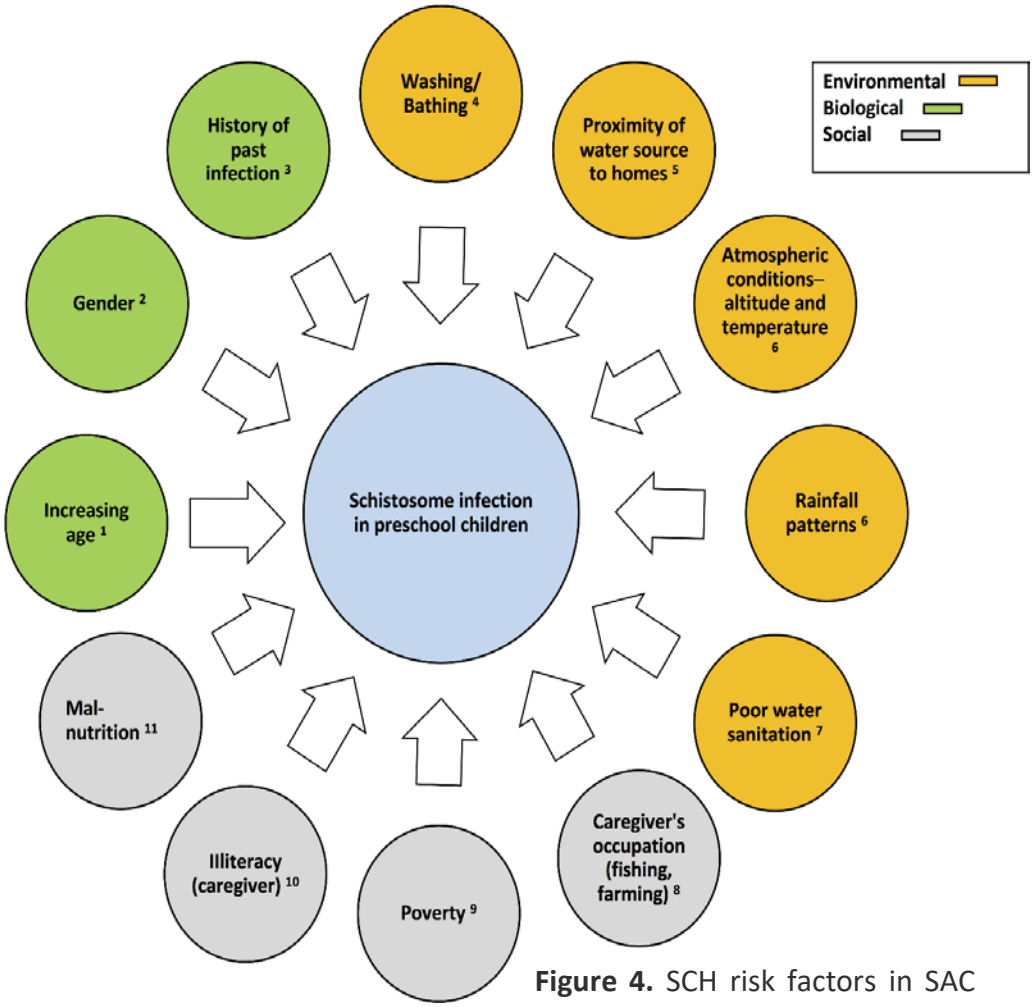

Figure 4. SCH risk factors in SAC

Health impact

Some health impact on PSAC includes anaemia, poor nutrition and growth (5). Even with low parasite burdens in PSAC, the pro-inflammatory response generated can quickly lead to chronic morbidity (36). The risk of malnourishment in endemic areas is related with host immune responses modulation by helminths, which has a negative impact on the host microbiome structure and nutrition (37). Urinary morbidity has been recently described in PSAC (38), as well as reduced vaccine efficacy at infancy (related with chronic exposure to schistosome infection) (39). Also, future cognitive deficits and educational lost was associated with schistosome infection (40). A Nobel prize recognizes deworming contributes to improving children's health and school performance and alleviating poverty (41). However, pathology and morbidity still remain poorly described and studied in PSAC (11).

Schistosome infection is also related with malaria severity and treatment. Studies in SAC suggest that malaria severity is compounded by schistosomiasis, and that malaria treatment is more effective when schistosome infection is also treated too (42) (43). In addition, *S. haematobium* infection has been shown to reduce the level of protective IgG responses to malaria vaccine candidates (44). However, these results need validation in PSAC.

Figure 5 shows an estimated proportion of morbidity attributable to *Schistosoma haematobium* infection in PSAC. The morbidity, reflected as microhaematuria, is an indication of active bladder and ureteral lesions and blood loss even in mild schistosome infection (34).

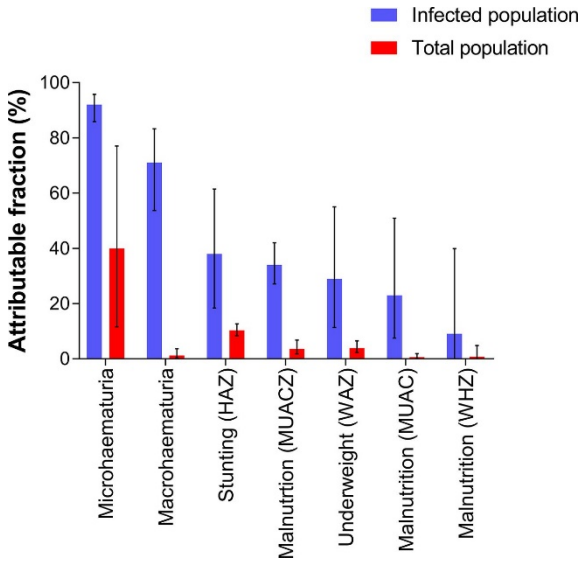

Fig 5. Estimated proportion of morbidity attributable to *Schistosoma haematobium* infection in the infected population (blue; AFe) and in the total population (red; AFp). Error bars indicate 95% CIs. BAZ, body mass index-for-age Z scores; HAZ, height-for-age Z scores; MUAC, mid-upper arm circumference Z scores; WAZ, weight-for-age Z scores; WHZ, weight-for-height Z scores. (17)

Treatment

PZQ, the anti-helminthic of choice for treatment of schistosomiasis, is safe and efficacious in PSAC (45). In this age range treatment can produce some benefits:

- Treatment of the first schistosome infection could have a longer-lasting impact on susceptibility to reinfection and presents the possibility of targeting treatment for maximum benefit in terms of the future health of the child. So there is a possibility where a single PZQ treatment can induce resistance to reinfection in PSAC (46).
- Also, it is shown in adults and SAC that treatment of schistosomiasis infections with praziquantel enhances schistosome-specific immune responses. This process occurs

by removing the immune-suppressive effects of the adult worms and introducing large amounts of parasite-specific antigens to the immune system to reach the threshold required to induce an immune response (11).

- A study shown that significant amount of morbidity, as measured by microhaematuria, resolved within 3 months of effective treatment with PZQ. Therefore, schistosome morbidity in PSAC can be reversed by PZQ treatment too (34).

In some situations, the prevalence of schistosomiasis infection in these young children is very high. Recent studies have shown that praziquantel, when made palatable for small children, is safe, well tolerated, and effective in the treatment of pre-school children. However, inclusion of very young children in mass treatment campaigns could prove disruptive and unsafe as there is currently no appropriate paediatric formulation of praziquantel. It is recommended that pre-school children should be treated for schistosomiasis within child-health services where their weight is monitored; they are immunized, dewormed and given micronutrient supplements (47). There's an operational model being proposed for the treatment of schistosomiasis in PSAC called ''diagnose and treat'', in contrast with mass drug administration (MDA) current model.

In the context of national control programmes and to speed administration of treatment, weighing scales (dose of at least 40mg/kg) have been replaced by a PZQ treatment or dosing pole that, for school age children, spans the corresponding doses of one to three tablets (in half-tablet divisions) across a child's height range from 94 cm to 160 cm(21).

This age group is an important target for treatment and whatever intervention that decrease child mortality, also contribute to the healthy development of children into adults able to reach their full potential (48). For that reason, WHO revision of the schistosomiasis treatment guidelines includes PSAC (31). Although, the true global infection and disease burden remains unknown (11).

ANGOLA

The Republic of Angola is an emerging country located at the Sub-Saharan region of Africa. Bordered by Democratic Republic of the Congo to the north, Namibia to the south, Zambia to the east, and the Atlantic Ocean to the west. It has a total population of 25,022,000 people and its capital and largest city is Luanda. Portuguese is the official language (49).

After a prolonged civil war (1975-2002) of 27-years, the standard of living remains low for most of the population, and the life expectancy is among the lowest in the world. It is the last in the top ten of higher infant mortality rate with 65.8 deaths per 1000 live births (50).

Fig. 6: Angola Map (49)

SCH epidemiology in Angola

Angola is third country in the rank of SCH prevalence after Kenya and South Africa with 6,436.27 cases per 100,100. It has the same position in reference of YLDs (Years Lived with Disability) with 45.33 per 100,000. As well as in DALYs (Disability-Adjusted Life Years) with 65.50 DALYs per 100,000. However, it is located in the second position in relationship with SCH death (0.55 deaths per 100,000), after Ghana (51).

It is an endemic country requiring PC. In 2017, 6,711,724 individuals in the country requiring PC for SCH annually, from which 3,851,818 are SAC. There were implemented 164 units for SCH. 46 of them were delivering PC for SAC however, only 28.6% were achieving effective (>= 75%) coverage of SAC. 1,734,872 individuals were receiving PC which suppose 25.8% of national coverage. Also, 1,724,711 SACs were receiving PC (44.8% national coverage) (52).

The province of Benguela which is formed by 10 municipalities (Cubal is one of them) is located in the west of Angola. In the territory of Benguela there are several water courses, belonging to the four hydrographic basins found in the province: Cubal, Handa, Catumbela and Coporolo. Other geographical features are the steppe formations found in the vegetation of the province area, as well as open forest formations and wooded savannas. These environments conditions are suitable for the colonization of molluscs and the formation of schistosomiasis foci.

Cubal municipality is located in province of Benguela with an estimated population of 322000 inhabitants, where nearly half of them are under 15 years of age. The rural area represents the 97% of the population, and villagers are mainly engaged in subsistence farming (53). Access to clean water and improved sanitation are lacking. The 67.8% of the population has not access to treated water and the just only the 12% has latrine (53) (which increase outdoors schistosome egg release). Also, some studies shown that there is 100% positivity for schistosomiasis in patients with clinical symptoms in the municipality. The area was selected for this study because the prevalence of *S. haematobium* is high (>50%), while the prevalence of *Schistosoma mansoni* and soil-transmitted helminths is low (<5%).

These evidences the severity of the disease in the studied region. It is necessary to provide urgent measures for treatment and prophylaxis due to the alarming prevalence of *Schistosoma haematobium* infestation (54).

WHO

Neglected tropical diseases (NTDs) are a group of parasitic and bacterial diseases that cause substantial illness for more than one billion people globally. Affecting the world's poorest people, NTDs impair physical and cognitive development, contribute to mother and child illness and death, make it difficult to farm or earn for living, and limit productivity in the workplace. As a result, NTDs trap the poor in a cycle of poverty and disease. There are 17 NTDs. The seven most common are (9): Dracunculiasis (Guinea Worm Disease), Lymphatic filariasis, Onchocerciasis, Schistosomiasis, Soil-transmitted Helminths (STH) (Ascaris, hookworm, and whipworm), Trachoma. To prevent, control, eliminate and eradicate of NTDs, WHO proposed for each one a few objectives to achieve in a certain period, which is called ''The roadmap''.

For SCH in 2012 (the roadmap proposed in the period of 2012–2020), the World Health Assembly adopted resolution WHA65.21 on the elimination of schistosomiasis, calling on all countries endemic for schistosomiasis to analyse and develop applicable plans with progressive objectives, to intensify control interventions towards elimination, to ensure the provision of essential medicines and WHO to elaborate a procedure to evaluate the interruption of transmission of schistosomiasis (55).

In the region of Africa, the proposed objectives were:

- Reach the goal of treating at least 75% of school-aged children in all endemic countries in Africa. This means 76 million school-aged children should receive praziquantel treatment.
- Eliminate *S. haematobium* in Zanzibar
- Interruption of the transmission of this infection in Mauritius

To achieve these objectives WHO recommends two main strategies among others: preventive chemotherapy (PC) and provision of safe water, sanitation and hygiene (55). Preventive chemotherapy is one of the main interventions used by national programmes to control the morbidity due to SCH. The intervention is distributed through mass drug administration (MDA) and school-based treatments, with the goal of treating populations at risk of infection at appropriate, regular intervals with praziquantel (56).

To estimate the population requiring PC for SCH annually, the following model is used (15):

- High risk area – all school-age children and adults required PC (e. g. Angola).
- Moderate risk area – 50% of school-age children and 20% of adults to be treated.
- Low risk area – 33% of school-age children to be treated. This is equivalent to treating school-age children twice during their schooling years.

* (Pre-SAC are not included in PC campaigns for schistosomiasis because there is no suitable formulation of praziquantel)

Globally preventive chemotherapy for SCH is required in 52 countries for a total of 219.9 million people: 120,3 million school-aged children (SAC) and 99.6 million adults (57). WHO African Region has the highest burden of schistosomiasis, with 90.4% of the people who require PC for SCH.

In 2017, more than 98.7 million people received free preventive treatment for schistosomiasis (81.1 million school-aged children and 16.9 million adults). In relation to the targets of the neglected tropical disease (NTD) roadmap, the coverage of SAC with PC was 68% for schistosomiasis (57). This shows encouraging trends towards the goal of attaining a minimum target of treating at least 75% of school-aged children in areas endemic, making it technically feasible to achieve the global targets set for 2020. (58).

Stringent efforts have been made to eliminate SCH through the implementation of sustainable control strategies. However, there's available evidence suggesting that both urinary and intestinal schistosome infection are still a matter of major public health concern and causing significant morbidity and there are disability in the endemic countries, particularly in SSA region (15).

JUSTIFICATION

Schistosome infection is still a matter of major public health concern and causing significant morbidity and disability in the endemic countries, particularly in SSA region. It affects around 240 million people every year, of which 123 million are children and at least, 50 million of them are PSAC.

PSAC is a high-risk group, with an important infection burden and it has been neglected without a place on schistosomiasis health programmes. For that reason, after WHO revision of the schistosomiasis treatment, PSAC has been included for their treatment on high endemic areas. However, there is a gap of information about prevalence, diagnosis and dose treatment in this group.

Furthermore UCP-LF-CAA has demonstrated to be a specific test to diagnose schistosome. Although, its cost is higher than gold standard test (urine filtration) and the necessity of laboratory tools, machines and cold chain (for keeping samples) makes it less appropriate in low source places. Nevertheless, the use of DBS as extraction sample tool and implementation of UCP-LF-CAA dry reagents might reduce the price and simplify the technique.

For those reasons, we propose this study to performance the use of UCP-LF-CAA (with dry reagents) in DBS and serum. Comparing CAA concentration for each one. We will proceed to make parasitological diagnosis to treat positive cases too. This will lead us to assess the prevalence of the disease.

HIPOTHESIS

Feasibility and reliability of CAA testing in DBS improves schistosome infection diagnosis in pre-school aged children.

OBJECTIVES OF THE STUDY

Main objective:

- To assess the correspondence between Serum and Dried Blood Spot results for Quantitation of Schistosoma Circulating Anodic Antigen.

Secondary objectives:

- To assess the prevalence of schistosomiasis *s. haematobium* in preschool-aged children in Cubal.
- To validate CAA testing in DBS collected onto Whatman 903 Protein Saver cards

MATERIALS AND METHODOLOGY

STUDY DESING

A diagnostic test study with a **cross-sectional** design carried out in Cubal city, the capital city of the municipality. Laboratory procedures will be performed in the Hospital Nossa Senhora da Paz in Cubal city.

STUDY AREA AND POPULATION

The study population will be preschool aged children (children under 5 years) living in Cubal city.

SAMPLE SIZE

The required number of individuals to detect significant differences in the diagnostic outcome was calculated with an equation given by Epidat 4.2. The sample size used for this study is based on the prevalence of 60% shown on SAC in previous studies in Cubal (59). The program takes into account an expected sensitivity: Test 1: 76,000%; test 2: 85,000% and expect specificity: test 1: 79,000%; test 2: 90,000%. Using a significance level of 5% and a power of 80%, the minimum required number of individuals that had to be examined was 506.

SAMPLE METHOD

A **consecutive non-probabilistic sampling** will be performed. Through popular games we will inform population and aim to participle in our study. People will be invited to Hospital Nossa Senhora da Paz where we will explain the study and the process, then, we will give them the informed consent. In case they accept, we will show when and how they should take the urine samples and we will meet the next days to perform the study.

ESTIMATE TIME FOR RECRUITMENT

Based on previous studies with similar sampling method we estimate 6 months for taking the sample. Nevertheless, we will extend this time to 8 months to ensure that even if any unexpected event occurs, we will have time enough to recruit the needed number of patients.

INCLUSION AND EXCLUSION CRITERIA

The study will enrol children who met the following inclusion criteria. Participants had to:

- Be younger than 5 years of age
- Written informed consent by parents/guardians (*Annex A*)
- Submission one urine sample for a 10 ml filtrate

- Adequate DBS collection sample

The exclusion criteria that not permit to participate in the study is:

- Have recent schistosomiasis treatment (within the past 6 months) according to a parental questionnaire.

VARIABLES OF THE STUDY

We define our variable as infection status (positive/negative) for schistosomiasis. It will be measure through **parasite diagnoses** (diagnose test recommended by WHO) and through **antigen detection**.

PARASITE DIAGNOSES

About 50 mL of urine sample will collect from each participant. Samples will be collected between 10:00 hours and 14:00 hours and will be processed within 2 hours of collection. For very young children, urine bags will used to collect urine. Urine samples were examined microscopically for *S. haematobium* infection following the standard urine filtration method (60) and the number of eggs will be reported per 10 mL of urine. Children will be diagnosed positive for helminth infection if at least one parasite egg will be detected in their urine samples.

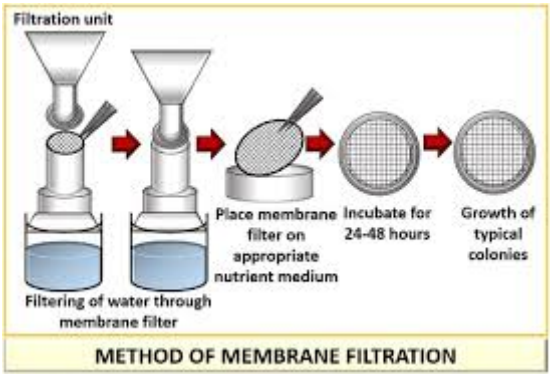

Fig.7: membrane filtration technique (61)

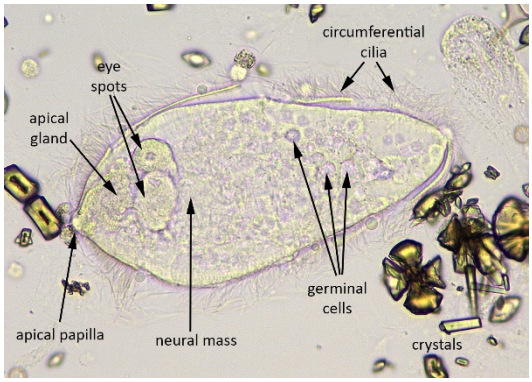

Fig.8: *s. haematobium* microscopy (62)

UCP-LF ASSAY

Results of UCP-LF assay for CAA in serum or in DBS will be positive or negative for schistosome infection depending on CAA concentration. Standards of known

concentrations, run with each assay, will be used to construct a standard curve and to determine the cut-off point above which samples will be considered positive.

Serum samples

Serum samples will be extracted by venepuncture. We will follow a standard procedure for the collection of blood specimens to get accurate laboratory results. All the steps will be procedure by an expert nurse or physician using paediatric sterilised material.

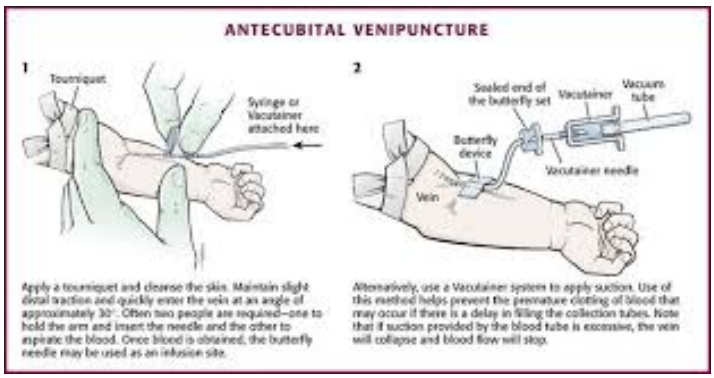

Fig 9: paediatric venepuncture (13)

DBS samples

DBS is a form of collection where patients place blood drops on a filter card after a finger prick with a lancet. Once dry, blood spot cards are extremely stable for shipment and storage (63). Each card should have the name and number to differentiate it.

In the reaction vessel, the dried blood is extracted from the material using a defined volume of sample buffer. The resulting extract corresponds to a diluted blood sample and can be applied in the respective ELISA or immunoblot system for the requested analysis.

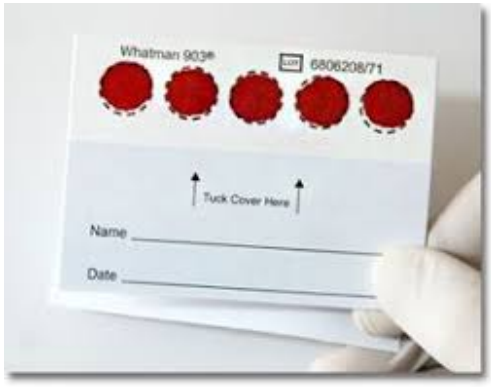

Fig 10: standardized collection card (64)

Fig 11: Sampling methodology (65)

Diagnostic assays built on Circulating anodic antigen (CAA) targeting require extraction of the sample with trichloroacetic acid (TCA) followed by centrifugation, which leaves the carbohydrate components in the TCA supernatant and precipitated protein material in a pellet. The advantage of the TCA extraction is an improvement of the analytical sensitivity. Assay sensitivity was further improved with the introduction of the up-converting phosphor (UCP) technology and switch from an ELISA-based platform to a LF-based platform. The UCP-LF assay for CAA detection was recently adapted to a dry reagent format that allows convenient storage at ambient temperature and worldwide shipping without the need for a cold chain (66). **Fig 19-20** show the steps in the procedure of UCP-LF CAA testing with dry reagents and how interpret the results (*Laboratory procedures*)

COVARITES

We will pick up other variables in order to ensure some of them will not be confounding factors or interactive variables. In case they were, we will control them to increase the external and internal validities of our study. We will able to minimize the effect of our confounding variables later, with a multivariate analysis. Most will be recollected by a questionnaire (*Annex B*) based PSAC schistosome infection study on Nigeria (67).

1. **Age**: measured in years. Patients younger than 5 years old. It can be subdivided into: At birth, until first year, 2-5 years.
2. **Sex**: female or male
3. **Parents' data:**
    - Basic data related with parents' occupations and highest level of education will be collected on the Questionnaire (*Annex B*). The level of education will be classified in: Primary, Secondary, Tertiary or No formal education.
    - Also, they will be questionnaire about their knowledge about schistosome infection symptoms.

4. **WASH-related variables**: Water, Sanitation and Hygiene. These variables are related with the prevalence of the infection. Its control on statistical analysis it important to calculate de predictive value of the test.

4.1 Main source of drinking water', was dichotomized into the categories 'safe' and 'unsafe', based on the suitability of the water source for accommodating the intermediate snail hosts. Safe water sources included wells and rainwater, while unsafe water sources included freshwater bodies, such as dams, lakes, and rivers.

4.2 Neighbour: Based on previous studies we will locate each house according to its proximity to water resources. The figure 6 shows the main water facilities nearby used by the local population: A pond in the northeastern area of the city, and the Cubal River, which crosses the city by its southern border.

4.3 Latrine usage was also dichotomized, separately grouping children who reported using a latrine at home or at their neighbours' homes and children who did not use latrines.

4. 5 Water contact: first contact, frequency of exposure and activities related with the water contact will be collected in the same questionnaire. First contact was classified as: at bird, before first year and 2-6 year of life. Frequency as: everyday, once a week and once a month. Finally, activities will be asked as: bathing, washing, recreational activities, fetching of water and others.

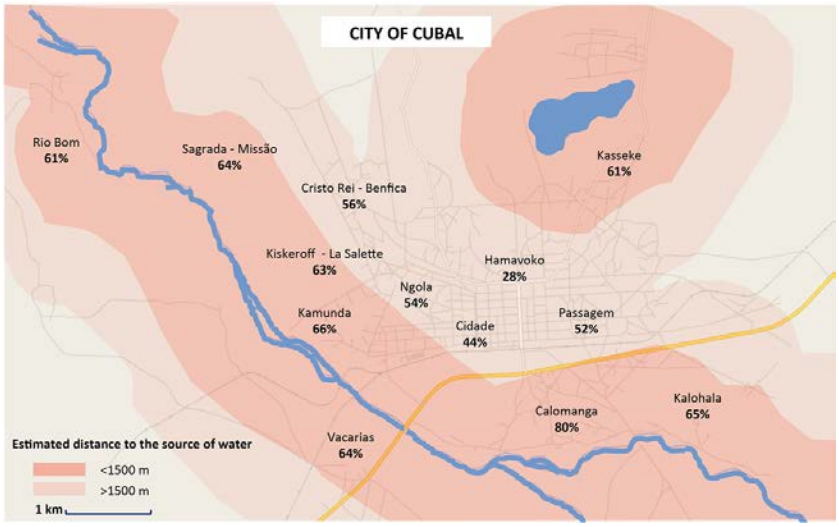

Fig. 12 main water facilities (59)

5. **Diseases:**

5.1 Previous schistosome infection: Previous schistosome infection and treatment will be asked with the questionnaire.

5.2 Other diseases: Through the questionnaire (*Annex B*) we will ask for Malaria, diarrhoea, eye diseases, respiratory infection, worms and abdominal problems. The answers will be yes, no or not sure for each disease.

6. **Anthropometrical measurement:** Although these variables will be collected in the questionnaire, each one need different performance, explained here.

6.1 Weigh: It will be measured without shoes and in light clothing using an electronic scale. For very young babies, younger than 2 years old or unable to stand, tared weighing will be done. In this second procedure, firstly the mother alone will step on the scale. Without moving out of the scale re-set the reading to zero, give her child to hold and the child's weight will appear on the scale.

Fig. 13 Weighing a child with an electronic mother/child weighing scale (68)

6.2 Length or Height: Depending on a child's age and ability to stand, will measure the child's length or height. Height is lower than length (the ligaments are opposed in standing position).

6.2.1 A child's **length** is measured lying down (recumbent) with an infantometer baby board. The length board should be placed on a flat, stable surface such as a table.

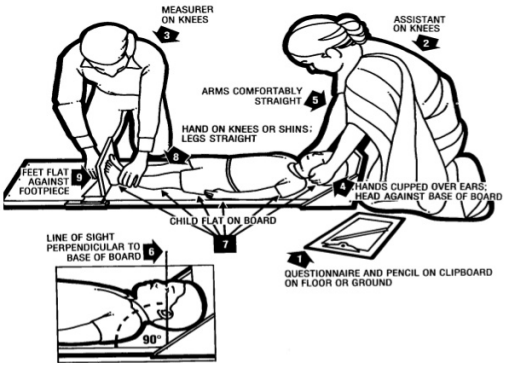

Fig 14 Infantometer baby board (68)

6.2.2 **Height** will be measure standing upright, without shoes and in light clothing using a stadiometer. The stadiometer will be mounted at a right angle between a level floor and against a straight, vertical surface such as a wall or pillar. It provides a direct read out of height with an accuracy of 1.0 percent.

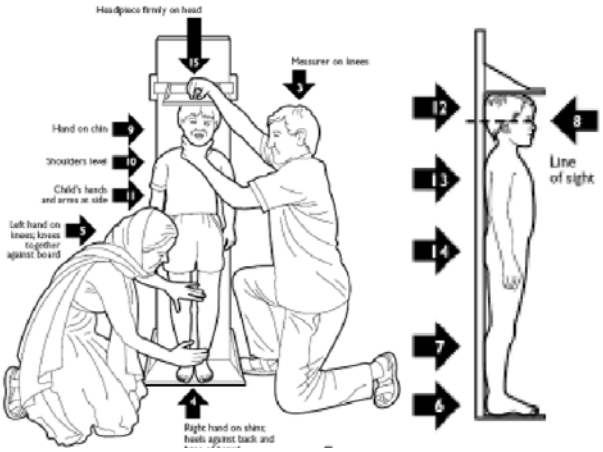

Fig. 15 Measuring the standing height of a child (68)

6.3 MUAC: The mid-upper arm circumference (MUAC) will be measured (nearest 1 mm) using a child MUAC tape; on the left arm, midpoint between the shoulder and the tip of the elbow, with the arm relaxed and hanging down the body. During 1-5 years of age it remains reasonably static between 15-17 cm among healthy children. If it between 12.5 and 13.5 cm it is indicative of moderate malnutrition. If it less than 12.5 cm it is suggestive of severe malnutrition.

Fig 16: Child MUAC tape (69)

Fig 17 Child MUAC tape colour code (69)

6.4 Nutritional status: Nutritional status will be determine using age and anthropometric parameters. It will be computed based on World Health Organization 2010 growth standards. Growth and nutritional status will be assessed using the WHO Anthro software V.3.0.1. This generated Z-scores for specific measures of nutrition and growth, that is, stunting by height-for-age (HAZ), underweight by weight-for-age (WAZ) and body mass index-for-age (BAZ) and malnutrition by MUAC (MUAC and MUACZ) and weight-for height (WHZ). Measures will be considered abnormal when Z scores were <–2.

7. **Haematocrit:** The haematocrit measures the volume of red blood cells compared to the total blood volume (red blood cells and plasma). The normal haematocrit for men is 40 to 54%; for women it is 36 to 48%. This value can be determined directly by microhematocrit centrifugation or calculated indirectly. Automated cell counters calculate the haematocrit by multiplying the red cell number (in millions/mm3) by the mean cell volume (MCV, in femtoliters). It will be measured in the blood sample.

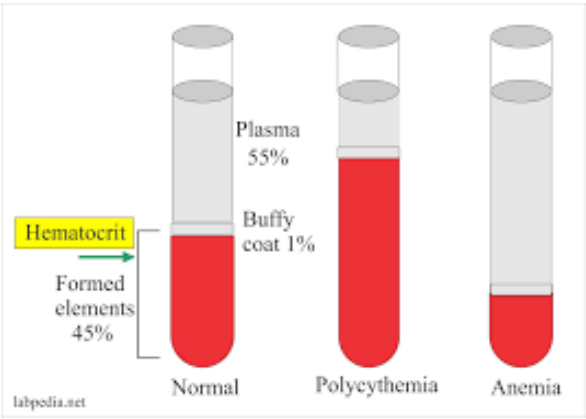

Fig 18: haematocrit (68)

PROCEDURES

The study will be composed by two parts. The first one will be in the field where we will collect all the data. The second one will be the laboratory procedures where we will observe the results of each test and perform the statistical analysis.

Field

1º After the sample collection, if the children meet the inclusion and exclusion criteria we will contact with each legal tutor, explained our study and distribute the parental consentient (*Annexe A*).

2º Once accepted, we will explain how they should take the urine samples (previously explained), and we will administrate the questionnaire (*Annexe B*). Some questionnaire items as weight, height, MUAC... will be collected in the next steps.

3º Next day we will recollect urine samples, blood samples and DBS samples. Additionally, children's height, weight and MUAC will be measured. All the samples will be examined in the Hospital Nossa Senhora da Paz's laboratory where they will performance the laboratory procedure diagnostic.

4º Anthropometric data will be analysed by WHO Anthro 2010 software to generate Z-score values.

5º All children who will be positive for *S haematobium* infection will be treated with a single dose of praziquantel (PZQ) at the standard 40 mg/kg bodyweight at each visit (weight will measure using an electronic scale. For very young babies, with a baby scale). Tablets were crushed and administered with squash and sliced bread by local nurses. Also, we will treat children with malnourishment.

6º Data will be analysed by the statistician anonymously, giving them the identification number of the patient

Dried blood spot sample preparation

In CAA test to elute dried samples from cards, we will cut sections from the DBS and placed them into Eppendorf's containing 100µL of phosphate-buffered saline. The sections will be incubated overnight at 4C, then placed on a shaker for 2 hours at 38C, after which 100µL of 4% (w/v) tricholoroacetic acid (TCA) will be added and the mixture vortexed and centrifuged. We will be eluted DBS with a total area of 144mm2 into final concentration of 2% TCA. The supernatant from these elution's will be concentrated to a final volume of 20-30µL using a 10kDa concentration device (Amicon Ultra-0.5ml Centrifugal Filters, Millipore Corp).

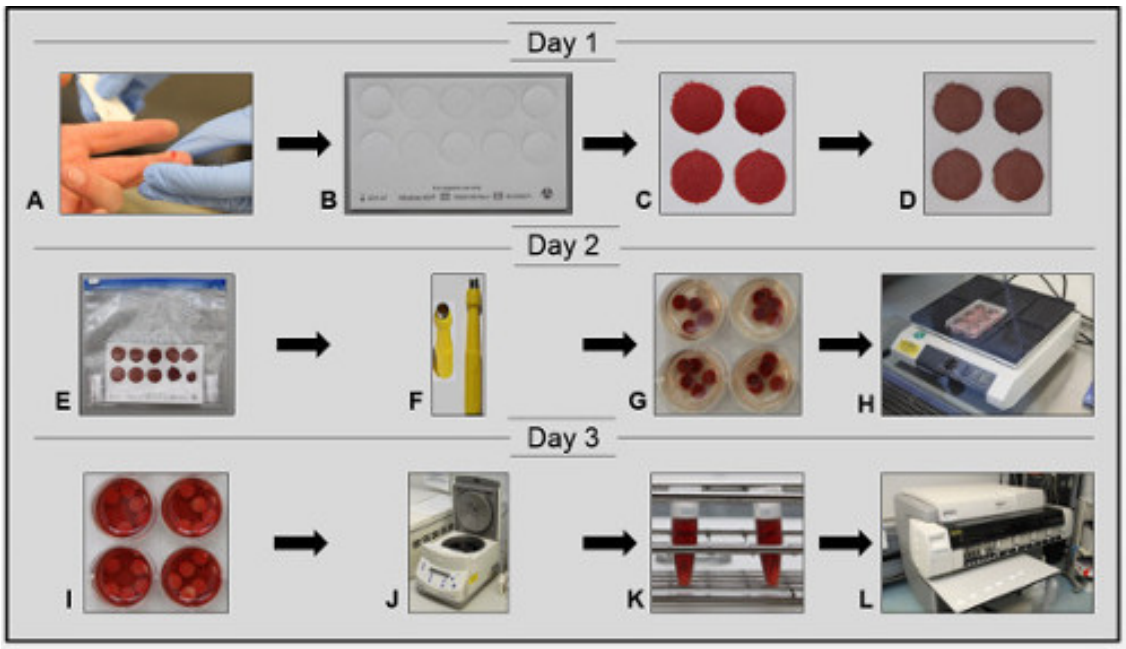

Fig 19: DBS sample preparation (70)

CAA test strip preparation and testing

Serum samples (20µL) will be mixed with an equal volume of 4% TCA, vortexed, and centrifuged. Twenty microliters of this supernatant, 20µL of the supernatant of the eluted samples from the small circles, or all of the concentrated eluate supernatants were subsequently mixed with assay buffer containing UCP reporter particles labelled with anti-CAA McAb, incubated, and applied to CAA-specific lateral flow test strips. The test line signal was normalized to the control line signal for each individual sample.

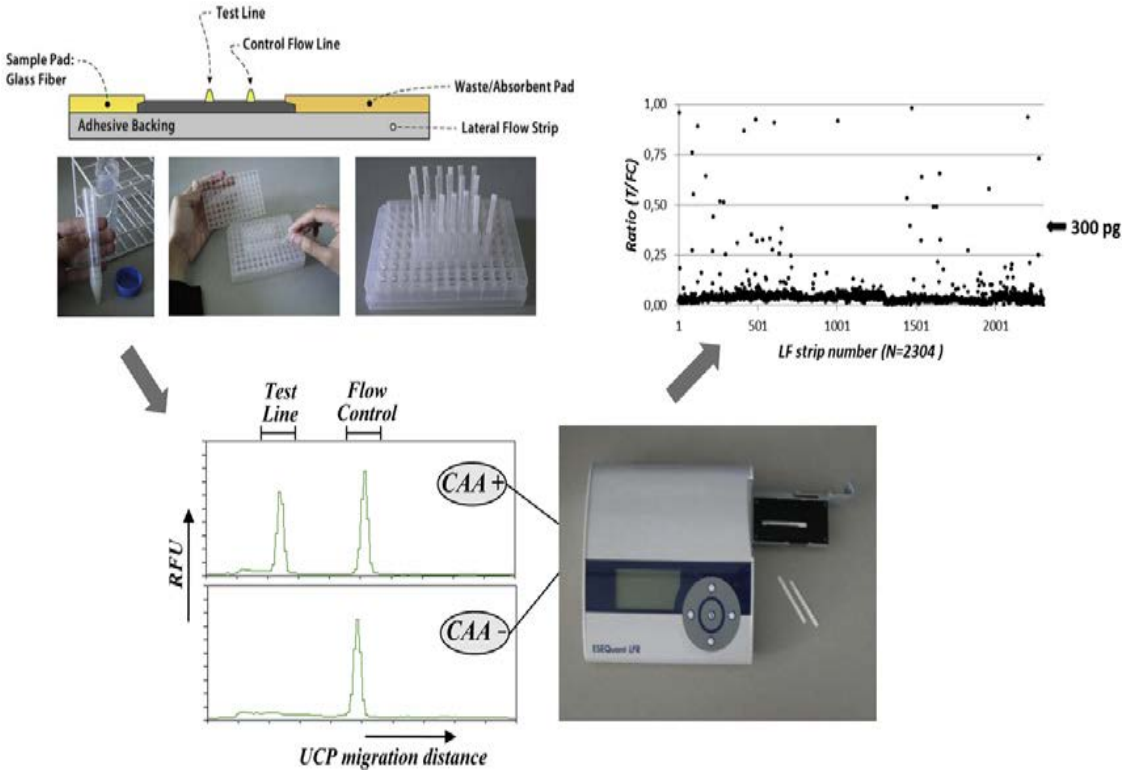

Fig 20: The CAA UCP–LF dry assay format and portable strip readers (72)

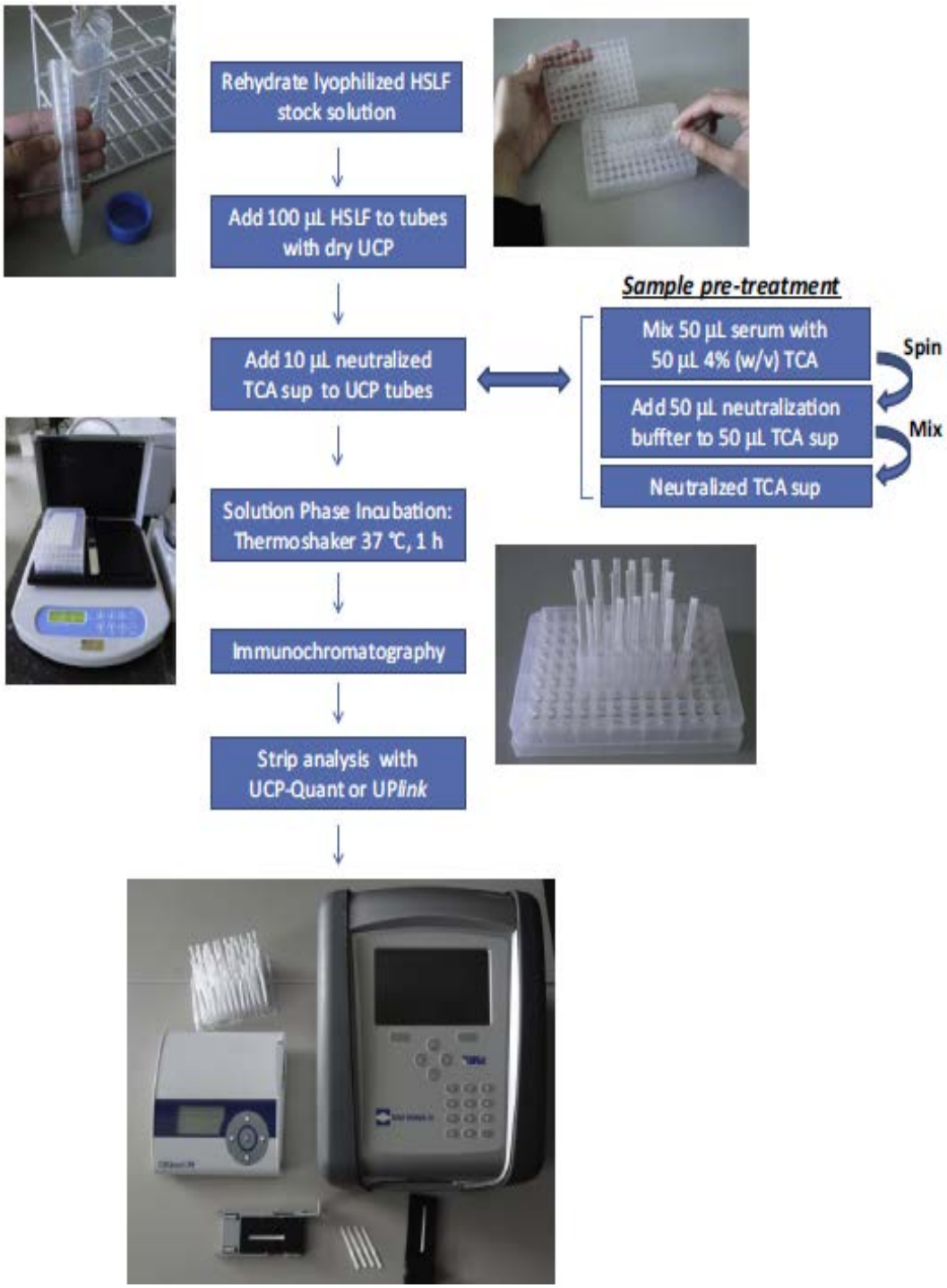

Fig 21: Steps of the procedure (72)

STATISTICAL ANALYSIS

UNIVARIATE ANALYSIS

The variables will be expressed as percentages for categorical variables (sex, infection status, nutrition status…) and as mean ± SD for continuous variables (age, height, weight…). If the continuous variable is not normally distributed, it will be express as median (quartiles).

BIVARIATE ANALYSIS

The primary end point of the study is to compare CAA in DBS and CAA in serum performance as a diagnosis tool using parasitological diagnosis as the reference standard. We will compare the infection status (positive/negative) with UCP-LF CAA in DBS and blood against the ‘‘gold-standard’’ diagnosis of schistosomiasis, recommended by WHO. Agreement between positive and negative test results will be assessed using Cohen's kappa coefficient.

To evaluate the validity we will generate sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for UCP-LF in DBS and serum samples. To compare DBS and serum, a contrast of binomial proportions will be performed, assuming normal distribution. A curve ROC will be represented for each one. To compare both curve ROC we will use a, Newson, R. B. Comparing the Predictive Powers of Survival Models Using Harrell’s C or Somers’ D. The Stata Journal. 2010;10:339–358.

As the importance of the test’s predictive value we will estimate schistosome infection prevalence. It will be calculated dividing positive individuals (diagnoses by the gold standard) with all the individuals in the sample.

STRATIFICATION OF THE RESULTS DEPENDING ON COVARIABLES

Having the results of this data we can stratify them by the covariables with the aim of detecting any interaction that may affect the conclusions of the study.

A multivariate analysis will be accomplished to adjust our variables for covariables; thus, we will try to avoid potential confounders that could modify the results. So, in order to analyse the relationship among our independent variable with the covariates, we will perform a multivariate regression logistic model.

ETHICAL CONSIDERATIONS

The research protocol should be presented, evaluated and submitted to the Clinical Research Ethical Committee (CEIC) at the Hospital Nossa Senhora da Paz in Cubal, Angola, before the beginning of the study.

This protocol will be carried out in accordance with the ethical principles established by the World Medical Association in the *Helsinki Declaration of Ethical Principles for Medical Research Involving Human Subjects* (last updated in October 2013). This study also will be carried out considering the basic principles established by: *The Ethics of Research Related to Health care in Developing Countries*. Nuffield Council on Bioethics (2005), *The Standards and Operational Guidelines for Ethical Review of Health-Related Research with Human Participants*. World Health Organization, WHO (Geneva, 2011) and *the International Ethical Guidelines for Biomedical Research involving Human subjects*. Council for International organization of Medical Sciences, CIOMS (Geneva, 2016)

According to "*Ley Orgánica 3/2018, de 5 de Diciembre, de Protección de Datos Personales y Garantía de los derechos digitales*", personal and clinical information of participants will be confidential and only used for the purpose of the research. Moreover, all data will be analysed anonymously. The participant (or the responsible person of the participant) will always be allowed to modify or destroy any of their collected data. The study will also have to follow the "Ley 14/2007, Real Decreto 1716/2011", which regulate research related to human health that involves invasive procedures.

Note that our study will also follow the " *Ley 41/2002 Básica Reguladora de la Autonomía del Paciente y de Derechos y Obligaciones en materia de información y documentación clínica*" about the basic regulation of the autonomy of the patient and of rights and obligations in the matter of information and clinical documentation. Thus, parents or guardians of all participating children must give informed consent, and must have had it signed before the children is included in the study. Information must be given both orally and written, in Portuguese and Bantu, and it must be confirmed that the information has been well received and understood through the interpreter.

Those responsible (parents or legal guardians) for the participants will be personally informed by researchers and an informative document about the study will be given to them, where they will find all the information of the process of data collection, measurement of anthropometry, the necessary additional tests (schistosomiasis; blood analysis, urine test) to which he/she should be submitting, and objectives of the study will be explained in detail. Finally, al the end of the study, all PSAC will be given crushed praziquantel tablets (40 mg/kg, against schistosomiasis) free-of change and irrespective of their infection status.

STUDY LIMITATIONS

In this study there are some potential limitations that have been contemplated to try to minimize them.

- To avoid selection bias caused by the sampling method, exclusion and inclusion criteria will be defined and patients who wish to participate in the study must fulfil them. The way we will enrol people in the study may decrease sample heterogenicity. Therefore, the sample will be less representative.
- In order to avoid water distance as confusion variable, a stratified sampling could be an interesting sampling. Although, due to lack of number of individuals by neighbourhood, we couldn't implement it. However, we take it into account as other confusion variable and analyse like that.
- Both the reference test and the test being evaluated must be applied and interpreted blindly and independently, to avoid diagnostic suspicion bias, so that knowledge of the results of a test can influence the interpretation of the other test.
- Adequate sampling should be performance to decrease information bias. To sterile and safety obtain blood samples in paediatric population a trained physician is necessary. DBS sampling, although seem easy, should be recollected in a correct way. In order to avoid inadequate samples, we will train team workers. Finally, to collect correctly urine samples, children tutors will be informed the best moment to do it.
- To decrease interobserver variability tests will be performance and interpreted by one expertise.
- As the Hospital Nossa Senhora da Paz is not completely computerized, many information will have to be stored in paper until the Forms are completed and data is dumped into a database. This can increase the possibility of information loss. The research team will be told about this issue to maximize the care and minimize the problem.
- Confusion variables will be avoided making multivariable analysis.

FEASIBILITY

The main researcher (MR) will be a physician specialise on infectious diseases with a great curriculum in the context of research who will coordinate the entire project; participate in the collection data; interpret the statistical analysis; write the final paper and present the results.

Another physicians, nurses and laboratory workers will participate.

The community health workers will be the connection with the field for our investigators, and will be the ones introducing us to the community and its understanding.

A qualified statistician (S) who will make the statistical analysis of the results.

WORK PLAN

STAGE 1: Protocol design (4 months)

This level consists on literature review for the elaboration of the study, the development of the protocol and subsequent presentation to the Ethical Committee of the Hospital Nossa Senhora da Paz in Angola for its approval.

- **Study setting-up**: During this period of time, the investigator and co-workers will make a literature review, propose objectives and hypothesis, develop a methodology and a draft of the protocol design.
- **First informative meeting**: When the draft of the protocol design has been made, it will be presented to the collaborators and they will agree with an execution plan and organization.
- **Final project design and writing**.
- **Protocol revision and approval**: The protocol will be presented to CEIC for its revision and approval.

The approximate duration of this stage will be about 4 months, varying according to the time that the Ethical Committee takes to approve the protocol.

STAGE 2: Preparation and initial coordination (3 months)

- **Second informative meeting**: Once the protocol had been approved by the CEIC, all the details of the project will be presented so that everyone knows what their role is within the study and a chronogram will be created to clarify the different phases of the study. All doubts that staff have will be resolved at this time. It will be programmed new meeting during the study to evaluate the problems that have been experienced until the moment and propose potential improvements. All the team will keep in touch via e-mail.
- **Research team training**: Laboratory staff will be trained on diagnostic procedures. Two training weeks will be necessary to prepare in the new techniques.

STAGE 3: Field work and data collection (12 months)

- **Sample collection:** During a recruitment period of 8 months a detailed work of sampling will be carried out meeting inclusion and exclusion criteria already specified.
- **Laboratory test**: Once the laboratory test and the evaluation will be performed, the data collected on the questionnaire presented in Annex B will be digitalized and saved waiting for future analysis. Any child that requires treatment, will begin to be treated, according to the protocols of WHO.

STAGE 4: Data analysis and article elaboration (4 months)

- **Data analysis**: A statistician will take all collected data and will proceed to analyse it with a specific statistical program.
- **Results interpretation**: With the statistical data obtained, the investigators will analyse and discuss about the collected data.
- **Final report writing**.

STAGE 5: Results publication and dissemination (3 months)

- **Results publication**: The results will be presented to specific national and international conferences and meetings.
- **Final report dissemination**: The final report will be submitted to scientific journals to be published.

STUDY CHRONOGRAM

ACTIVITIES	PERSONEL	2020												2021												2022	
		J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F
STAGE 1 Protocol design																											
Study setting-up	MR																										
First informative meeting	ART																										
Final project design and writing	ART																										
Protocol revision and approval	CEIC																										
STAGE 2 Preparation and initial coordination																											
Second informative meeting	ART																										
Research team training	ART																										
STAGE 3 Field work and data collection																											
Sample collection	ART																										
Laboratory test	ART																										
STAGE 4 Data analysis and article elaboration																											
Data analysis	S																										
Results interpretation	MR																										
Final report writing	MR + S																										
STAGE 5 Results publication and dissemination																											
Results publication	MR																										
Final report dissemination	MR																										

*MR: main researcher

ART: all research team

S: Statistical

BUDGET

Many of the activities in this study will not generate any cost as bibliography research and protocol design. Also, materials already available at the hospital will not be included. Therefore, for this study we are expecting to need:

PERSONNEL

- **Two Community Health Workers** will be given an extra salary of 100€ per month, in the 8 months that they will be needed that is 800€ per each one, 1,600€ in total
- Three **laboratory technicians** will be given an extra salary of 200€ per month, in the 8 months that they will be needed, that will be 1,600€ each one, 4,800€ in total.
- Two **nurses** enrolled at the team, in charge of taking measures and collecting samples, will be given an extra salary of 300€ per month, in the 8 months that they will be needed, that will be 2,400€ each one, 4,800€ in total.
- Two **interpreters** will be needed for the nine months of recruitment and will receive 150€ per their work, 1,350€ each one, 2,700€ in total.
- **Statistician** 72 x 35€/h in total, 2,520€ is the estimated cost for this service

MATERIAL AND SERVICES

- DBS cost approximately 1.50€ each.
- Developed CAA concentration protocol adds to the cost of the UCP-LF CAA assay (3.00€/test)
- Urine test will cost 2.5€

Total cost per child included in our study: 7€; The number of children studied will be 506. TOTAL cost: 3,550€

An average treatment is estimated to be between 0.20–0.30€ for each PZQ-treated child (2.2 tablets per child) (21)(71)

PUBLICATION AND DISSEMINATION

The publication budget has been estimated as well as the assistance of the research team to conferences and congresses (registration, transport and accommodation included).

- The publication in a scientific journal will cost 1.500€ approximately.
- With the idea of disseminating knowledge, we will go to the next International Nutrition Congress, which will cost about 2.500€ taking in account the travel, the inscription, accommodations, etc.

The total cost of publication and dissemination will be 4,000€

OTHER JUSTIFIED COSTS

Questionnaire and informed consent print 0,15 € 506 units, 76€

	Price per unit	units	TOTAL
PERSONNEL			
Community Health Workers	800€	2	1,600€
Laboratory technicians	1,600€	3	4,800€
Nurses	2,400€	2	4,800€
Interprets	1,350€	2	2,700€
Statistician	35€/h	72h	2,550€
MATERIAL AND SERVICES			
DBS	1.50€	506	760€
UCP-LF CAA assay	3€	506	1,518€
Urine test	2.5€	506	1,265€
Treatment	0.30€	506	152€
PUBLICATION AND DISSEMINATION			
Publication in journals			1.500€
Attendance to congresses			2.500€
OTHER JUSTIFIED COSTS			
Questionnaire and informed consent print	0.15€	506	76€
TOTAL			24,221

CLINICAL AND HEALTH CARE IMPACT

The demonstration of schistosome CAA quantitated in DBS stored on Whatman 903 Protein Saver cards correlated with serum values, has important implications for clinical and health care.

First, the ability to test for CAA in DBS represents a streamlined way in which schistosome testing can be enhanced. Schistosome infection diagnosis sensibility and specificity will be increased. Also, it will be adapted to PSAC, finding a safety way to take samples on paediatric population.

Second, our work could create an opportunity to enhance both patient care and clinical research by coupling schistosome testing with HIV- or malaria-related testing in the same DBS.

In addition, the ability to test for CAA in DBS will represent a streamlined way in which schistosome testing can be improve, either on its own or in concert with other projects, thereby increasing attention to this neglected parasitic infection.

ANNEXES

ANNEXE A: INFORMATION SHEET AND CERTIFICATE OF CONSENT

This informed consent form is for the parents of children younger than 5 years of age who attend the hospital Nossa Senhora da Paz, and who we are asking to participate in the research about improvement of schistosome infection diagnose.

Principal investigator identification:

This Informed Consent Form has two parts:

- Information Sheet (to share information about the study with you)
- Certificate of Consent (for signatures if you agree that your child may participate)

You will be given a copy of the full Informed Consent Form

PART I: Information Sheet

Introduction

We are doing research on schistosomiasis, which is very common in this country I am going to give you information and invite you to have your child participate in this research. You do not have to decide today whether or not you agree that you child may participate in the research. Before you decide, you can talk to anyone you feel comfortable with. There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask them of me, the study doctor or the staff.

Purpose

Urinary schistosomiasis is a disease produced by *s. haematobium* which in children principally produces microhaematuria, anaemia, poor nutrition and growth. They have higher vulnerability, low infection with less diagnoses because of the low sensitive and specificity of the gold standard. UCP-LF CAA has been demonstrated as useful diagnosis test. The purpose of this research is to assess the correspondence between Serum and Dried Blood Spot results for Quantitation of Schistosoma CAA on UCP-LF. Also, to assess the prevalence of schistosomiasis *s. haematobium* in preschool-aged children in Cubal. We will also write down clinical and analytical data, in order to improve in the future

acknowledgement and treatment of these patients. These can help us in the future to improve the diagnose of schistosomiasis in children.

Type of Research Intervention

Assess three diagnostic tests for schistosomiasis with different samples, and take note for clinical and analytical variables.

Participant selection

Schistosome infection has really importance in children under 5 years old. Chronic exposition is related with urinary morbidity and future cognitive deficits and educational lost. The infection can impair they normal growth and modify immune responses as well as reduced vaccine efficacy. Also, its prevalence in this age period is unknown.

We are inviting you to take part in this research because it is important to know schistosomiasis diagnose as it can be treated decreasing the impairments commenting above. We can only do it in children that are under this situation, as your child does.

Voluntary Participation

Your decision to have your child participate in this study is entirely voluntary. It is your choice whether to have your child participate or not. If you choose not to consent, all the services you and your child receive at this hospital will continue and nothing will change. You may also choose to change your mind later and stop participating, even if you agreed earlier, and the services you and/or your child receives at the hospital will continue.

Description of the Process

You may stay with your child during the visits and the procedures. In the visit, two small amounts of blood, equal to about a teaspoon, will be taken from your child's arm. Your child will feel some discomfort when the needle stick goes into her/his arm but this will go away very quickly. There may be slight bruising but this will disappear in a few days. In the same visit we will take a fingerstick sample and a urine sample (brought from home). The doctor will ensure that your children receive the best care and will ask you and him/her some questions about possible symptoms, and also will make a review of

the children in order to find some signs that will be written down. The same day of your visit we will measured and weight your children.

Two days after the first visit, you will have a little follow up, just to check the result of the test that we did on the arm of your children. You can ask questions in any moment and for any procedure. All the samples taken from your child will help us to know whether your child is infected with schistosomiasis (parasites) and he/ nutritional status. If you don't want us to do some of the above let us know. Because all the named before is important for the study, if we cannot assess these tests, we will not be able to include you in this study. You will be informed about all the results involving your children's health. There are guidelines to follow to treat and manage the conditions found in your children, and we will follow them for her/his best assessment.

The biological samples obtained during this research procedure will be used only for this research, and will be destroyed after 2 years, when the research is completed.

Duration

The research takes place over 2 years and 2 months in total, from which 8 months we will be taking information about children like yours. During that time, we will test the samples taken from your child and you will be informed of any medical condition that we found. If any treatment is needed you will be asked to come to the hospital for the correct management.

Side Effects

The interventions are not expected to have any side effects or risks, but if something happens, your children can come to the hospital and the health professionals will do the better treatment for your children, and will carry with the money burden of this management.

Discomforts

By participating in this research, it is possible that your children may experience some discomfort such as the discomfort of the injections. There may be a slight hardening and/or swelling where the needle stick goes into the skin. This should disappear in one day. If you are concerned, please call me or come to the hospital.

Benefits

If your child participates in this research, he/she will have the following benefits: any interim illnesses will be treated at no charge to you. If we detect any of the associated conditioned mentioned above, your child will be treated at no charge at you, and you will also be offered to be tested and treated for this disease. There will be benefits to the society as children health implies better future of the society.

Confidentiality

The information that we collect from this research project will be kept confidential. Information about your child that will be collected from the research will be put away and no-one but the researchers will be able to see it. Any information about your child will have a number on it instead of his/her name. Only the researchers will know what his/her number is and we will lock that information up with a lock and key. It will not be shared with or given to anyone except the health care workers, nurses, doctors or researchers that needed it for assess her/him specific information.

Sharing of the results

The knowledge that we get from this study will be shared with you before it is made widely available to the public. Confidential information will not be shared. There will be small meeting in the community and these will be announced. Afterwards, we will publish the results in order that other interested people may learn from our research.

Right to Refuse or Withdraw

You do not have to agree to you child taking part in this research if you do not wish to do so and refusing to allow your child to participate will not affect your treatment or your child's treatment at this Hospital in any way. You and your child will still have all the benefits that you would otherwise have at this hospital. You may stop you child from participating in the research at any time that you wish without either you or your child losing any of your rights as a patient here. Neither your treatment nor your child's treatment at this Centre will be affected in any way.

Who to Contact:

If you have any questions you may ask them now or later, even after the study started. If you wish to ask questions later, you may contact with the number and/or e-mail of the nutritional service of the hospital, or came to the hospital, and they will put you in contact with the main researcher.

PART II: Certificate of Consent

Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily for my child to participate as a participant in this study.

Print Name of Participant_____

Print Name of Parent or Guardian_____

Signature of Parent or Guardian _____

Date _____

Day/month/year

ANNEXE B. QUESTIONNAIRE

QUESTIONNAIRE FOR RESEARCH ON: Schistosomiasis, intestinal helminthiasis and Nutritional status in Infants and pre-school aged Children in Communities

Community _____Lat. _____ Long. _____ Altitude_____

General information of parent/caregiver Age _____Sex_____ Occupation_____

Duration of stay in community: (a) < 5 years (b) 5-10 years (c) >10 years

Education: (1) Primary (2) Secondary (3) Tertiary (4) No formal education

Do you have a generating set? (a) Yes (b) No

KNOWLEDGE ABOUT URINARY SCHISTOSOMIASIS AND ITS TRANMISSION

1. How many pre-school children do you have? _____

S/No	Identification	Sex	Age (DOB)	Dirty fingernails?	Trimmed fingernails?
a.					
b.					
c.					

2. Have you heard about the word "urinary schistosomiasis"? (a) Yes (b)No

3. Has any of your wards/children has schistosomiasis before? (a) Yes (b)No

4. a). How do you know that your child or ward has urinary schistosomiasis? _____

b). what are the symptoms?_____

5. Have any of your children ever gone/exposed to water resources?

(a)Yes (b) No (c) Don't know

6. If yes, which of them? (a) Preschool aged (b) School aged

7. When was the first exposure? (a) At birth (b) before first year (c) 2-6 year of life

8. If at birth, how? (a) Baby was taken to stream (b) water from stream used to bath child at home. (c) Other

9. If during first year of life, how? (a) Child taken to stream (b) water from stream used to bath child at home (c) other

10. If 2-6 years of life, how? (a) Child taken to stream by parents (b) water from stream used to bath child (c) child goes to stream by himself

11. What activity exposes your ward to the river water? (a)Bathing (b) Washing (c) Recreational activities (d) fetching of water (e) others_____

12. What is the frequency of exposure to stream/river water of preschool children? (a) Everyday (b) once a week (c) Once a month

13. What have you done about the infection? (a) Nothing (b) Taken to the hospital (c) Taken to herbalist

14. Do you think that infection is normal? (a) Yes (b) No

15. What do you suggest can be done to effectively control urinary schistosomiasis infection?

16. Does any of your wards passed out blood in their urine in the last 3 weeks? (a) Yes (b) No

17. Does any of your ward experience painful urination? (a) Yes (b) No

18. What is the distance to the nearest health centre from your household? (a) Far (b) Near

NUTRITIONAL ASSESSMENT

S/ No	Identification	Sex	Age (DOB)	Height (cm)	Weight (kg)	Mid-upper arm circumference
a.						
b.						
c.						

Assessment of Household Sanitation Facilities

1. What kind of toilet facility do you use? (a) Water Closet (b)Pit with slab (c)Open pit latrine (d)Bush (e) River
2. Do you share your toilet? (a) Yes (b)No If yes how many households? ____
3. Is the toilet facility located within the premises? (a) Yes (b)No
4. How far is the toilet facility from the kitchen/room (a) Near (b) Far
5. What is your source of water for domestic use? (a) Tap (b) River Ogun (c) Well (d)other

SECTION C - Assessment of Habit

1. Do you have home slippers? (a)Yes (b)No
2. How often does your ward wear slippers/sandals/shoes? (a)Don't wear (b)Always (c)Seldom
3. How do you clean your ward's hands after defecation? (a)Water (b) Paper/Leaves (c)Water + soap (d) none
4. Does your ward pick food from the ground? (a) Yes (b)No
5. Does your ward have any record of swimming/wading/baptism in water? (a) Yes (b)No

For Infants:

1. How often do you breastfeed your child per day? (a) Thrice (b)4-5 times (c) > 5 times
2. What is the average duration of a single breastfeed? (a)2 minutes (b)5 minutes ( )> 5 minutes ( )
3. What is your child's other food supplements? _____
4. What hygiene practice do you observe before breastfeeding your ward?_____

6. Do you wash your hands before you feed your child? (a) Yes (b)No

For Older pre-school children

1. Do you wash your ward's hands before eating? (a) Yes (b)No
    a. If yes, what do you use? (a)Water (b)Water + Soap
2. At what age did you normally stop breast-feeding your child?_____
3. Observe if PSAC has dirty fingernails? (a) Yes (b)No
4. Observe if PSAC has trimmed fingernails? (a) Yes (b)No

SECTION D - Assessment of Health History

1. Has your ward had any history of stomach pain? Yes ( ) No ( ) Not sure ( )
2. Has your ward had history of diarrhoea/dysentery? Yes ( ) No ( ) Not sure ( )
    a. If yes, when was it? (a) this month (b) last month (c) more than 2 months ago
    b. How long did it last? (a) less than one week (b) 1-2 weeks (c) more than a week
3. Has your ever notice your child pass out worms? Yes ( ) No ( ) Not sure ( )
    a. If yes, at what age was the first time? _____
4. How often do you deworm your ward? Always ( ) Rarely ( ) Never ( )
    a. Have you deworm your ward this year? (a) Yes (b) No
    b. If yes, how many times? _____
5. Has your ward had any history of malaria? Yes ( ) No ( ) Not sure ( )
6. Has your ward had any history of respiratory infections? Yes ( ) No ( ) Not sure ( )
7. Has your ward had any history of eye diseases? Yes ( ) No ( ) Not sure ( )

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