Fast and reliable easy-to-use diagnostics for eliminating bilharzia in young children and mothers: an introduction to the freeBILy project

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Fast and reliable easy-to-use diagnostics for eliminating bilharzia in young children and mothers: An introduction to the freeBILy project

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ABSTRACT

Schistosoma antigen detection tests have a large potential for schistosomiasis control programs due to their ability to detect active and ongoing *Schistosoma* infections, their much higher sensitivity compared to microscopical methods, and the possibility to use non-invasive urine samples. Pregnant women and young children could especially benefit from affordable and easy-to-use antigen tests as inclusion of these vulnerable groups in mass drug administration campaigns will always require higher justification hurdles, especially in low to middle endemic regions with a higher proportion of individuals who are not infected and thus unnecessarily exposed to praziquantel.

The overall objective of the '**f**ast and **r**eliable **e**asy-to-use diagnostics for **e**liminating **bil**harzia in **y**oung children and mothers' (freeBILy, www.freeBILy.eu) project is to thoroughly evaluate the point-of-care circulating cathodic antigen (POC-CCA) and the up-converting phosphor reporter particle, lateral flow circulating anodic antigen (UCP-LF CAA) urine strip tests to diagnose *Schistosoma* infections in pregnant women and young children and to assess their potential as a schistosomiasis control tool in test-and-treat strategies. The freeBILy project will generate valuable, evidence-based findings on improved tools and test-and-treat strategies to reduce the burden of schistosomiasis in pregnant women and young children.

1. Background

Public health interventions and control efforts for schistosomiasis have primarily targeted school-age children and adolescents through mass drug administration of praziquantel (PZQ) without prior diagnosis (WHO, 2013). Even though control efforts were expanded to pregnant and lactating women, infants and preschool-age children (Stothard and Gabrielli, 2007; Johansen et al., 2007), these vulnerable groups are still regularly excluded from treatment programs, resulting in an increasing risk for schistosomiasis morbidity as well as continuing transmission of schistosomiasis (Freer et al., 2018; Luty and Elliott, 2016). Women in child-bearing age suffer two-fold from *Schistosoma* infections: directly from pathological effects of the worms and indirectly from potential adverse birth outcomes (i.e. low birth weight, preterm delivery and maternal anemia) when they become pregnant (Friedman et al., 2018; Barrion and Voss, 2013; Colley, 2014). Evidence supporting the use of PZQ treatment during pregnancy as being safe and efficacious is growing (Friedman et al., 2018; Olveda et al., 2016; Ndibazza et al.,

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2010), opening up opportunities to no longer deprive pregnant women from schistosomiasis treatment and control (Luty and Elliott, 2016; Friedman et al., 2018). Furthermore, the availability of a pediatric formulation of PZQ will be crucial to fully include infants and preschool-age children in MDA campaigns (Reinhard-Rupp and Klohe, 2017; Stothard et al., 2013).

However, even with a growing body of evidence supporting safety, PZQ mass treatment in these particular groups will always require higher justification hurdles, especially in the low to middle endemic populations in which a higher proportion of individuals is not infected and who would therefore be unnecessarily exposed to PZQ (Freer et al., 2018). Administration of PZQ to these vulnerable groups should preferably be limited to only those who have a confirmed *Schistosoma* infection, for example by means of a test-and-treat strategy. Unfortunately, established diagnostic methods such as microscopic egg detection in stool or urine or the presence of hematuria are not very sensitive and even less sensitive in preschool-age children (Nausch et al., 2014; Stothard et al., 2011). Furthermore, microscopic methods are cumbersome and are unattractive for mass screening concepts. Other easy-to-use, non-invasive diagnostic tools are needed to accurately detect ongoing active *Schistosoma* infections. Especially for vulnerable populations, the use of such tools in a cost-effective test-and-treat strategy would be ideal.

Detection of *Schistosoma* antigens such as the 'circulating cathodic antigen' (CCA) and the 'circulating anodic antigen' (CAA) for diagnosing *Schistosoma* infections is a concept reaching back into the 1970's (Bergquist, 2013). Both antigens, CCA and CAA, are constantly regurgitated by live *Schistosoma* worms into the blood stream of the hosts' circulation (van Dam et al., 1996), they are well cleared into the urine (van Lieshout et al., 2000) with little day-to-day variations (Polman et al., 1998). Both CCA and CAA have been shown to clear within a few days or weeks after PZQ treatment (Kildemoes et al., 2017; Sousa et al., 2019). However, several studies have demonstrated that PZQ treatment might not always be efficacious as individuals remain CCA- or CAA-positive after treatment (Kildemoes et al., 2017; Mwinzi et al., 2015; Hoekstra et al., 2020; Mishra et al., 2019) and in some studies the prevalence could be further decreased by providing another treatment (Mwinzi et al., 2015; Hoekstra et al., 2020). This has also been observed when using egg microscopy to measure efficacy of PZQ treatment (King et al., 2011; Munisi et al., 2017; Nalugwa et al., 2015; Kabuyaya et al., 2018). Diagnostic test platforms utilizing urine are available that allow screening for active ongoing *Schistosoma* infections at the point-of-care level (POC-CCA) or via the laboratory-based, up-converting reporter particle technology based, lateral flow (UCP-LF) CAA test (Corstjens et al., 2014; van Dam et al., 2004).

The user-friendly, field-applicable and low-cost commercially available POC-CCA test has demonstrated a better sensitivity compared to the Kato-Katz method. It is used for diagnosing intestinal schistosomiasis (Mwinzi et al., 2015; Colley et al., 2013; Coulibaly et al., 2013; Adriko et al., 2014; Tchuem Tchuente et al., 2012), while being less accurate for diagnosing urinary schistosomiasis (Midzi et al., 2009). Some studies have shown possible cross-reactivity with urine from pregnant women (Greter et al., 2016; Marti et al., 2020) as well as with samples from individuals experiencing urinary tract infections or haematuria (Rapid Medical Diagnostics, 2019). This false-positivity is likely attributed to the structure of CCA which contains repeating Lewis-X units, structures which can also be found on human anti-inflammatory cells (Polman et al., 2000; Van Dam et al., 1994). The interpretation of the POC-CCA test, in particular in case of faint lines (the so-called 'traces'), often remains a challenge (Peralta and Cavalcanti, 2018; Casacuberta-Partal et al., 2019; Bärenbold et al., 2018). Despite these limitations, the test has been studied extensively and is accepted widely as a valuable field-applicable diagnostic tool and is now being recommended for mapping prevalence of intestinal schistosomiasis and for surveillance purposes, as it is more sensitive and user-friendly for the detection of *S. mansoni* infections than Kato-Katz (Bärenbold et al., 2018; WHO 2020; WHO 2019; Knopp et al., 2013).

The ultra-sensitive and highly specific UCP-LF CAA test is a genus specific test which identifies active *Schistosoma* infections of any known species, including all veterinary ones. It has demonstrated excellent performance in detecting the four major *Schistosoma* species (*S. mansoni, S. haematobium, S. japonicum* and *S. mekongi*) in different endemic (Clements et al., 2018; Corstjens et al., 2020; Corstjens et al., 2015; Knopp et al., 2015; van Dam et al., 2015; van Dam et al., 2015; Vonghachack et al., 2017) and non-endemic settings (van Grootveld et al., 2018; Langenberg et al., 2020), including performance in near elimination settings (Balahbib et al., 2017; Gaspard et al., 2020). By using an ultra-sensitive reporter technology (up-converting reporter particles, UCP) in combination with LF immunochromatography and a unique monoclonal antibody, CAA concentrations can be measured in various sample types such as dried blood samples (Downs et al., 2015), plasma (Stete et al., 2018), serum (Corstjens et al., 2020) and urine (Corstjens et al., 2020; de Dood et al., 2018). Currently, the UCP-LF CAA test is laboratory-based, as it includes a sample pre-treatment step which requires some basic lab equipment. Utilizing a sample concentration step, the sensitivity of the UCP-LF CAA test can be increased to the level of detecting single worm infections (van Dam et al., 1996; Corstjens et al., 2020; Wilson et al., 2006). The ability of applying large sample volumes also makes the UCP-LF CAA test suitable for pooled sampling strategies (Corstjens et al., 2017).

Both CCA and CAA antigen detection tests are of importance for control programs due to their higher sensitivity compared to microscopy, hence providing more accurate information regarding prevalence but also worm-burden in active ongoing *Schistosoma* infections. Moreover, the tests are user-friendly as they can apply non-invasive urine samples. Before antigen testing can be applied to vulnerable groups such as pregnant women, mothers and young children, the diagnostic accuracy in these groups must be properly determined. This would require data from clinical and research trials, however, in general vulnerable groups are often excluded from such studies. Also, to correctly determine the efficacy of chemotherapeutics and to reduce the number of study participants and thereby costs, the availability of highly accurate diagnostic methods is essential. Currently, clinical studies on schistosomiasis still suffer from imprecise and low sensitivity egg detection methodologies; egg counts are highly variable due to the intrinsic limitations of sampling (e.g. circadian fluctuation of egg release, dependence on urine production and hence fluid balance, clustered egg distribution in stool (Doehring et al., 1983; Doehring et al., 1985; Engels et al., 1997; Engels et al., 1996)). Because CCA and CAA are constantly regurgitated by *Schistosoma* worms directly into the blood stream of the hosts' circulation and cleared into the urine with little day-to-day variation, they are ideal markers for detecting active infections with schistosomes including young worms. CCA and CAA levels decline rapidly after PZQ treatment and are therefore also suitable markers for assessing drugs or vaccine candidate´s efficacy.

2. The freeBILy project

The overall objective of the '**f**ast and **r**eliable **e**asy-to-use diagnostics for **e**liminating **bil**harzia in **y**oung children and mothers' (freeBILy, www.freeBILy.eu) project is a thorough performance evaluation of CCA and CAA antigen tests for the diagnosis of *Schistosoma* infections in vulnerable populations of pregnant women and young children in order to reduce schistosomiasis related morbidity in these specific groups. Both groups have been omitted so far from control programs mainly due to the risk of unnecessary exposure to PZQ and a lack of safety data. By applying adequate and sensitive diagnostics and provide treatment to those with a confirmed *Schistosoma* infection, morbidity due to schistosomiasis will be reduced in these groups.

Within the freeBILy project, two prospective clinical trials are conducted in two countries (Madagascar and Gabon) with different schistosomiasis endemic settings and with different objectives and

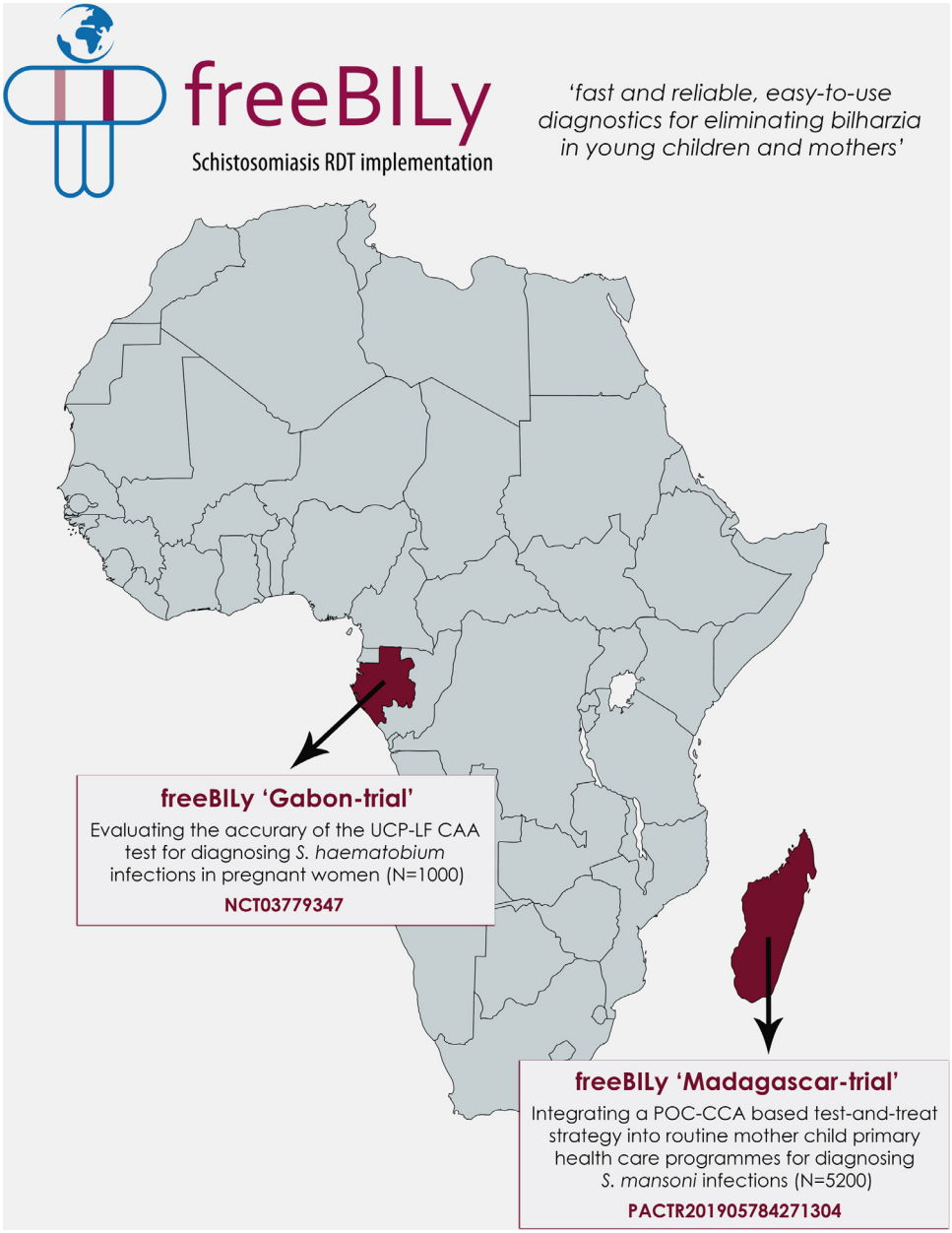

Fig. 1. freeBILy map showing the two different clinical trials.

endpoints, see Fig. 1. The freeBILy 'Madagascar-trial' will implement and evaluate the POC-CCA test in routine mother child primary health care programmes in *S. mansoni*-endemic regions in Madagascar. The freeBILy 'Gabon-trial' will focus on a comprehensive performance evaluation of the UCP-LF CAA test to diagnose *Schistosoma* infections and to monitor the efficacy of PZQ treatment in pregnant women in Gabon, where *S. haematobium* is predominantly prevalent.

2.1. freeBILy Madagascar-trial

The primary objective of the Madagascar-trial is the implementation of a POC-CCA test-based-schistosomiasis-treatment (TBST) into routine mother child primary health care programs at 'Centres de Santé de Bases' (CSBs) in Madagascar to demonstrate its usefulness for controlling intestinal (*S. mansoni*) schistosomiasis in pregnant women and young children. Secondary objectives include: (i) evaluating the performance of the POC-CCA test and investigating the cost-effectiveness of the POC-CCA TBST strategy on child development in comparison with the standard primary health care approach; (ii) to investigate the tolerability of PZQ use in pregnant women and newborns; (iii) to report the prevalence of *S. mansoni* infections in pregnant women and newborns in the selected study regions.

To address these objectives, a two-armed (intervention arm vs a control arm without intervention), 40-cluster randomized phase III trial (N = 5200 mother-child pairs) is conducted in two areas of medium to high *S. mansoni* endemicity in Madagascar: the regions of Antananarivo and Fianarantsoa. The units of randomization are primary health care centers (CSBs). In the CSBs included in the intervention arm, a POC-CCA TBST will be implemented. The two timepoints for TBST are at recruitment (5-6th month of pregnancy) and 9 months after delivery for both mother and her newborn. Collected urine samples will be tested on-site at the CSBs using the POC-CCA applying a visual scoring aid (G-scores) to allow for semi-quantitative scoring (potentially related to worm burden) and to ensure consistency in interpretation of the results and performance of the test (Casacuberta-Partal et al., 2019). In case of a positive POC-CCA test, PZQ treatment will be offered as part of the

TBST strategy. In the CSBs included in the control arm, samples will be collected but no TBST will be carried out and patient care will follow the national guidelines. At the final time point, 24 months after delivery, TBST will be also applied to the control arm. A separate cross-sectional study will be carried out in two additional CSBs to thoroughly investigate the performance of the POC-CCA test against other diagnostics for the diagnosis of *S. mansoni* infections in pregnant women and newborns. Samples will be collected from pregnant women and their newborns and tested by several different diagnostic tests including stool PCR (Meurs et al., 2015), serology and the UCP-LF CAA test (Corstjens et al., 2014; van Dam et al., 2013).

The primary outcome of the Madagascar-trial is children's height for age and maternal hemoglobin levels at 24 months of age for the newborns from the pregnant women included in the study. Accuracy of the POC-CCA test in pregnant women and newborns will be validated extensively in the cross-sectional study by comparing results against a panel of references tests, including microscopy, UCP-LF CAA, molecular tools, and serology. To evaluate the cost-effectiveness of the POC-CCA TBST strategy, information on resources used and associated cost for the POC-CCA TBST and for standard primary health care approaches is collected in a sub-set of CSBs. Health related quality of life information is also collected from a sub-set, both in the intervention and control arms, and will constitute the main health metrics component in the cost-effective analysis. The Madagascar-trial is registered at the Pan African Clinical Trial Registry under PACTR201905784271304.

2.2. freeBILy Gabon-trial

The primary objective of the Gabon-trial is to systematically evaluate the performance of the laboratory-based UCP-LF CAA test for *S. haematobium* detection in pregnant women. The Gabon-trial includes two additional sub-studies to further address related secondary objectives: i) assessing CAA as an endpoint measure of efficacy for future drug or vaccine trials in schistosomiasis which provides PZQ safety data for administration during pregnancy; ii) estimating PZQ efficacy in pregnant women in the *S. haematobium* region. Additionally, prevalence of schistosomiasis in pregnant women and newborns in Lambaréné and surrounding villages will be reported.

To address these objectives the Gabon-trial is organized into a set of three interlinked, prospective, observational studies which will be carried out in antenatal care centers (ANC) in Lambaréné and surrounding villages. The main study (study A) is a cross-sectional, diagnostic study evaluating the performance of the UCP-LF CAA test for diagnosing *S. haematobium* infections in pregnant women ($N = 1000$) who present at one of the ANC's somewhere in their 16^{th} to 30^{th} week of pregnancy. Urine and serum samples will be collected to determine the accuracy of UCP-LF CAA for the detection of *S. haematobium* infection in urine based on the correlation against a panel of reference diagnostics (microscopy, serology, molecular tools, POC-CCA). In observational study B, upon voluntary consent a minimum of at least 100 infected pregnant women from study A are included in an assessor-blinded study. Participants will be randomized into receiving PZQ treatment during pregnancy (early treatment group, $N \geq 75$) or no treatment during pregnancy (late treatment group, $N \geq 25$). The latter group is the control group which will receive PZQ treatment following national treatment guidelines after delivery. During a follow-up period of 6 weeks urines will be collected and tested weekly in order to determine the CAA levels over time and to estimate PZQ efficacy in this target population. In study C (observational, longitudinal), a subset of a minimum of 200 mother-child pairs from study A and B are followed up quarterly for a total period of 24 months to determine PZQ safety in mothers as well as maternal and birth outcomes, and the incidence of *S. haematobium* infections in the newborns. The Gabon-trial is registered at clinicaltrials.gov under NCT03779347.

3. Expected impact

The freeBILy project ultimately aims to clear the path for test-and-treat strategies for schistosomiasis control in pregnant women and young children, in order to reduce schistosomiasis related morbidity and on a long-term scale also mortality in these vulnerable populations. The ideal test for such a strategy would be an easy-to-use, rapid non-invasive diagnostic test, with appropriate sensitivity and able to identify all *Schistosoma* species. The freeBILy project will provide valuable information on the performance of the POC-CCA and the UCP-LF CAA urine tests in a new test-and-treat approach. The outcomes are also expected to provide new insight on previously observed challenges concerning the use and application of the field friendly POC-CCA test, i.e. the specificity in case of pregnancy and the interpretation of 'trace' findings (Peralta and Cavalcanti, 2018; Casacuberta-Partal et al., 2019; Bärenbold et al., 2018).

Capacity building is also major aspect of the freeBILy project. Local infrastructures at the African partner institutes will be upgraded. Study specific standard operating procedures will be developed including quality assurances procedures. Another important component is the implementation of the high sensitive UCP-LF CAA test format into the laboratories of the two respective African countries, which includes extensive training of and supervision of laboratory technicians. This will allow in-country validation of *Schistosoma* infection even in case of extreme low worm burden and thus omit the need for shipping samples out of the country to another centrally located test facility.

The clinical trials performed in Madagascar and Gabon will be implemented into routine mother child primary health care programs which consist of pregnancy care and routine child examinations and vaccination. By implementing a POC-CCA based TBST strategy into existing health care structures we will be able to generate reliable recommendations. Country specific Ministries of Health are involved via their National Control Programs (NCPs) in all aspects of the trials. These NCPs will facilitate access into the community and coordinate community awareness.

To improve the health of pregnant women and their children in schistosomiasis endemic regions, freeBILy outcomes will be disseminated by educating pregnant women on the disease, its health effects and how to diagnose and prevent the disease as well as by adapting schistosomiasis control policy towards a test-and-treat approach based on the outcome of the project. The expected benefit from treating schistosomiasis in vulnerable groups, pregnant women and young children, outweighs the relatively low risk that comes with PZQ treatment. With a test-and-treat approach the inclusion of these groups in MDA campaigns becomes a realistic goal (Bustinduy et al., 2016; Bustinduy et al., 2017). Furthermore, by identifying *Schistosoma* infections in children at a young age and treating them accordingly, the negative effect on their health, growth and cognitive development can possibly be reduced. These children will benefit through a better development in their younger years, which will continue in adolescence. In lower endemic areas, test-and-treat strategies could possible replace MDA campaigns.

The freeBILy project will provide important evidence-based findings on improved tools and test-and-treat strategies to reduce the burden of schistosomiasis in pregnant women and young children, which has been previously neglected but is certainly needed to eventually eliminate schistosomiasis as a public health problem.

Author contributions

GJvD, PTH, AK, NGS - Conceptualization of the freeBILy project. AAA, AK, NGS, RR, RAR, MRA, ES, PLAMC, GJvD and PTH - Funding acquisition, Conceptualization of the freeBILy clinical trials, Methodology. PTH - Writing original draft. All authors reviewed and approved the final manuscript.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2020.105631.

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