1 Objectives and End Points

Objectives

The overall purpose of freeBILy in Madagascar is to integrate a POC-CCA test-based schistosomiasis treatment (TBST) into routine maternal and child primary health care programmes.

The study investigates the effectiveness of the strategy for controlling schistosomiasis in young children and mothers.

The primary objectives were:

1. To assess the impact of TBST on child development by comparing schistosomiasis-associated growth disadvantage among young children in TBST sites (intervention) and in non-TBST sites (control).

2. To examine the impact of TBST on maternal health by comparing changes in hemoglobin levels (Hb) among pregnant women in TBST sites to those in non-TBST sites

2 Methods

Study design

Freebily study was a two-arm cluster randomized phase III trial, with clusters being primary healthcare centers called Centres de Santé de Base (CSB). A total of 40 clusters were included in this study. These 40 study centres are located in areas of medium to high endemicity of S. mansoni in Madagascar with 20 sites in the Itasy and Bongolava regions west of Antananarivo and 20 sites in the Amoron'i Mania region north of Fianarantsoa (Figure 1).

FreeBILy was implemented into routine maternal and child primary health care programmes, which consist of ante- and post-natal care, child routine examination and vaccination. In Madagascar, these programmes are established at CSBs, which are at the lowest administrative level of the health care system providing high accessibility to the population.

The study centers were randomly assigned to one of two arms:

1. TBST (intervention)
2. Standard of care (control).

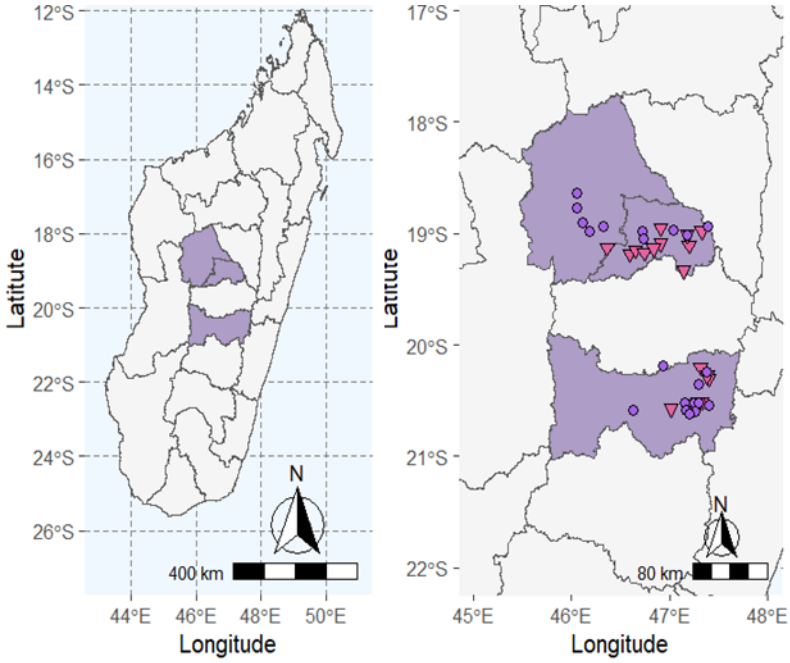

Figure 1. Distribution of the FreeBILy study sites across the regions of Bongolava, Itasy and Amoron'i Mania in Madagascar.

Intervention

In study centres of the intervention arm, the POC-CCA TBST was being implemented among pregnant women between the 5th month and delivery and at 9 months after delivery and among their babies at 9 months of age. The urine-based POC-CCA test was done, and PZQ treatment was offered in case of a positive test result.

Control arm

The control arm is the standard of care. In the study centres of the control arm, no TBST was carried out, and in case of clinical suspicion of schistosome infection, the participants were referred to the local health system. At the 24 months' time point, TBST was applied in both arms.

Randomization

Randomization was performed at the CSB level. A list with unique identifiers of the 20 CSBs in the regions of Bongolava and Itasy (so-called Antananarivo sites) and 20 CSBs in the region of Amoron'I Mania (so-called Fianarantsoa sites) was shared with the Leiden University Medical Center (LUMC), where half of the sites were randomly allocated to either control and intervention arm stratified by region. The randomization sequence was subsequently shared with the University of Antananarivo, University of Fianarantsoa and BNITM. While blinding at the CSB level was not possible due to the nature of intervention, all procedures of the study and of TBST (including urine sampling) are implemented at both the intervention and control CSBs. Only the actual POC-CCA test and the treatment of positives are exclusively done in intervention sites. In the participant ID, an alphanumeric element will define the recruitment site of the women. In case women would change the study site during the course of the study, the study nurses would be able to identify the non-correspondence of the site from the participant ID. This ensures that the randomization scheme is kept.

Figures 2 summarizes the study design

Eligibility criteria

The study parget population included pregnant women and their children.

Inclusion criteria:

- Informed consent signed (from parents for minors)
- Expected residency in the area for the study site for the next 24 months
- Pregnant women between their 5th and 6th month of pregnancy
- Children born from an enrolled mother, including twins
- Willingness to comply with the protocol requirements including sampling and treatment for both mother and children

Exclusion Criteria:

- Fever (temporary exclusion)
- History of transfusion
- History of congenital anemia
- Epileptic or convulsive episodes
- Non-pregnant women
- Pregnant women younger than 16 years old
- Pregnant women who did not complete their 4th month of pregnancy
- Pregnant women who do not live in the catchment area of the CSB
- Children born from mothers not enrolled in the study
- Children born in a CSB different from the ones in which the mothers were enrolled

Sample size

The sample size was calculated in relation to the primary child outcome proportion showing stunted growth at 2 years of age, for which a larger sample size is assumed to be necessary than for the maternal outcome . Calculations were performed using OpenEpi software. The following assumptions were made for the sample size estimation:

- Prevalence of stunted children at the age of 2 years in the population20%
- The intervention can reduce the proportion of stunted children by 50% in infected individuals, i.e. to observe that 10% of all children at the age of 2 years are stunted in the intervention arm.
- Significance level of 5%
- Statistical power of 80%
- Design effect of 1.9
- Annual loss to follow-up of 10% from a visit to visit
- Infant mortality rate of 4%
- Expected prevalence of schistosomiasis in pregnant women of 25%

Based on these assumptions the estimated required sample size was 4200 eligible pregnant women, an average of 105 women per cluster.

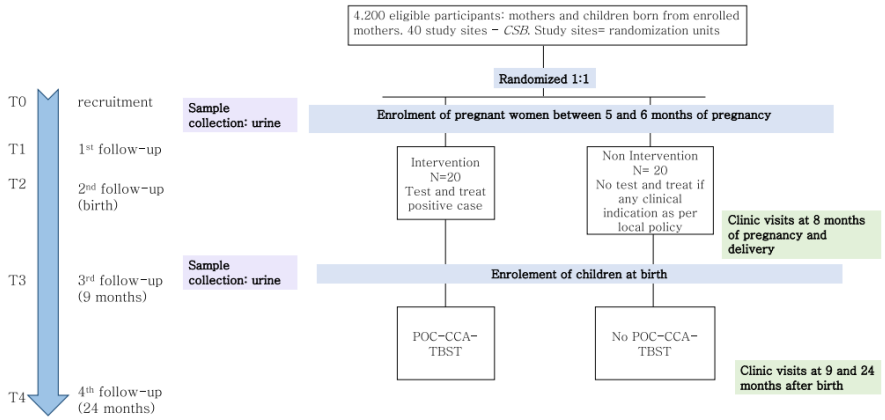

Figure 2. FreeBILy study: Cluster randomized trial design.

Data collection plan

The freeBILy includes 5 different visits (T0, T1, T2, T3, T4). All sites have the same visit schedule. The baseline visit was conducted following informed consent and eligibility assessment.

A POC-CCA-TBST was scheduled in the intervention arm at the following:

1. Recruitment (5th to 6th month of pregnancy) and 9 months after delivery for the pregnant women/mothers
2. Nine months of age for the children

Figures 3 summarize the data collection plan. Variables collected at each visit are summarized at Annex 1.

Scheme of scheduled visits			
Type of activity	Subjects	Intervention	Control
Visit	women	T0, T1, T2, T3, T4	T0, T1, T2, T3, T4
	children	T2, T3, T4	T2, T3, T4
Measurement of maternal Hb	women	T0, T1, T3, T4	T0, T1, T3, T4
	children	no visit	no visit
POC-CCA	women	T0, T3, T4	T4
	children	T3, T4	T4
Treatment of positive cases	women	T0, T3, T4	T4
	children	T3, T4	T4
Weight and height measurements	women	no visit	no visit
	children	T3, T4	T3, T4

Sampling and testing scheme							
Type of test	Type of sample	Subjects	T0	T1	T2	T3	T4
UCP–CF antibody assay[19]	Serum	women	1000	0	0	0	0
		children	0	0	0	0	0
Maternal Hb	Capillary blood	women	5200	5200	0	4472	3900
		children	0	0	0	0	0
UCP–LF CAA [20,21]	Urine	women	5200	0	0	0	0
		children	0	0	0	4472	0
POC–CCA [11]	Urine	women	5200	0	0	4472	3900
		children	0	0	0	4472	3900
PCR [22]	Plasma	women	1000	0	0	0	0
		children	0	0	0	0	0
PCR [23]	Stool	women	1000	0	0	0	0
		children	0	0	0	0	0
Microscopy [24,25]	Stool	women	500	0	0	0	0
		children	0	0	0	0	0

Figure 3. Freebily: scheme of scheduled visits and samples collection

Endpoints

The primary endpoints of the study are the following:

1. The proportion of children showing stunted growth (height-for-age z-value < − 2) at 2 years of age to measure the impact of TBST on child development
2. Change in levels of maternal Hb between recruitment and 8 months of pregnancy to measure the impact of TBST on maternal health

Data analysis

The primary analysis was conducted using intention-to-treat principles at individual level. As a sensitivity analysis, we restricted the intervention group to effectively treated.

Descriptive analysis

The descriptive analysis of baseline characteristics of study participants was conducted by the study arm at the enrollment of pregnant women (T0) and of children (T4) using the absolute frequency and the proportion for the categorical variables and the mean and standard deviation or median and interquartile range for numerical variables.

Analysis of the primary endpoints

For the main child endpoint, the proportion of stunted children (height-for-age z-value < − 2) at 2 years were compared between the intervention and control arm using mixed-effects poisson regression model with logarithmic link function to account for the between-cluster variation as a random effect.

To evaluate the impact of TBST on the change in hemoglobin levels and anaemia at the individual level we used a generalized linear mixed effects models. We adjusted the models for potentially relevant explanatory variables.

To characterize the safety profile of PZQ we estimated the proportion of severe adverse events in the intervention and control arms. Proportions were compared using exact Fisher test or chi-squared test.

3 Results

Exclusion flowchart

The exclusion of participants for the primary endpoint of pregnant women is presented below

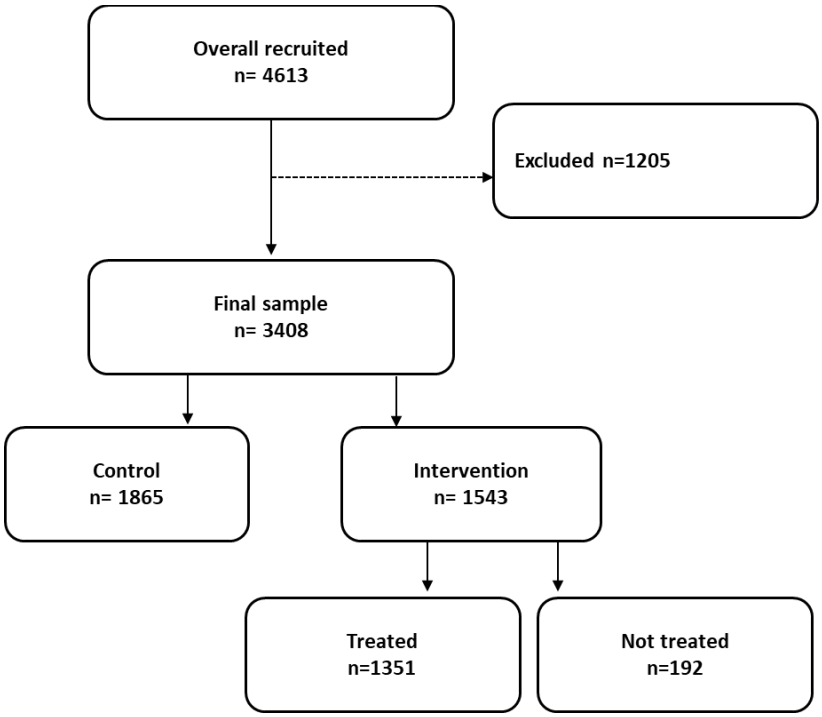

Figure 5. Recruitment flowchart pregnant women

The reasons for the exclusion of participants were the following:

- Age missing or below 16, n=2;
- Week of pregnancy out of range (<21 or >=29), n=138;
- T1 visit earlier than 4 weeks after T0, n=17;
- Withdraw n=73;
- Missed visit=949;
- Hb measurement is not available at T1, n=26.

Overall, of 5114 approached participants the final sample included 3408, 1543 in the intervention arm (TBST) and 1856 in the control arm.

Figure 6 shows the recruitment and exclusion flowchart for the primary endpoint analysis in children. Overall, of 4644 children born, 2862 were included in the study, 1224 in the intervention arm and 1638 in the control arm.

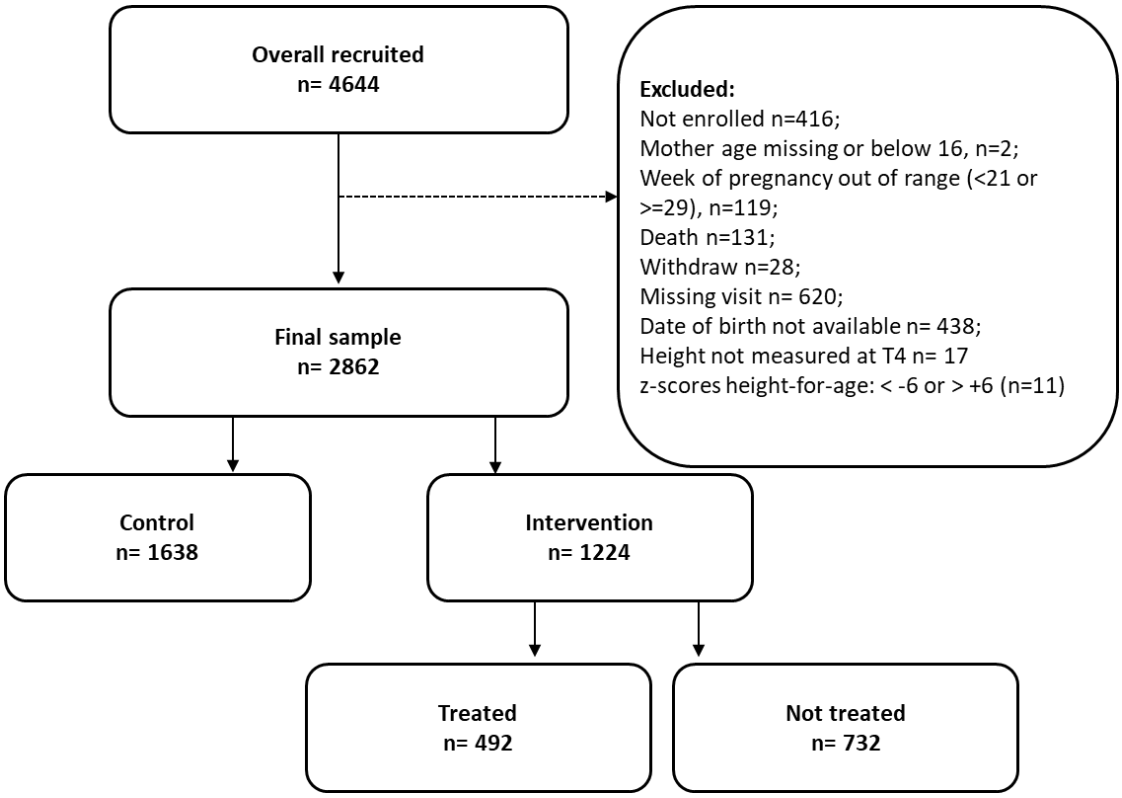

Figure 6 Recruitment flowchart children at T4

Descriptive statistics

At baseline distribution of participants by age group, education level, and contribution to a family income was similar between the standard of care and TBST groups. Differences were observed regarding the distribution of participants by region, urbanization, altitude and occupation (Table 1, Table 2) .

The proportion of participants recruited in per-urban settings was higher in the standard of care group as well as the proportion of those who reported farming as a primary occupation. In addition, participants from TBST group were recruited from locations with lower altitudes.

Regarding medical history, the proportion of women with Haemoglobin, leukocytes, and Protein in urine was similar between the standard of care and TBST groups. No differences were observed at baseline at participants' weight and systolic blood pressure and uptake of malaria preventive treatment during pregnancy.

In contrast, statistically significant differences were observed regarding previous history of treatment with PZQ, with a smaller proportion of the standard of care group (8.7% vs. 13.0%) reporting being ever treated. The proportion of supplement uptake (Folate, Iron) and treatment with Albendazole, also varied by group, being higher among TBST participants.

Table 1: Baseline characteristics of women at T0 per study arm

	TBST		**Standard of care**		p-value
	n	**%**	**n**	**%**	
Urban					0.0000
Rural	1208	78.3	1266	67.9	
Peri-Urban	335	21.7	599	32.1	
Region					0.0000
Amoron'iMania Region	780	50.6	860	46.1	
Bongolava	398	25.8	91	4.9	
Itasy	365	23.7	914	49.0	
Age group					0.0745
16-20	476	30.8	506	27.1	
21-25	466	30.2	583	31.3	
26-30	333	21.6	408	21.9	
31+	268	17.4	368	19.7	
Education level					0.0933
No formal education	58	3.8	64	3.4	
Primary school	701	45.4	917	49.2	
Secondary or more	784	50.8	884	47.4	
Occupation					0.0000
Not farmer	610	39.6	423	22.7	
Farmer	931	60.4	1442	77.3	
Main contributor to income					0.8792
Participant	41	2.7	48	2.6	
Other	1502	97.3	1817	97.4	

Albendazole treatment					0.0000
No	877	56.8	1330	71.7	
Yes	666	43.2	526	28.3	
Ever PZQ treatment					0.0001
No	1322	87.0	1680	91.3	
Yes	198	13.0	160	8.7	
Supplement: iron					0.0000
No	749	48.5	1167	62.6	
Yes	794	51.5	698	37.4	
Supplement: folate					0.0000
No	745	48.3	1167	62.6	
Yes	798	51.7	698	37.4	
Anti-malaria prophylaxis					0.7891
No	1073	69.5	1289	69.1	
Yes	470	30.5	576	30.9	
1st pregnancy					0.0589
No	1006	65.2	1273	68.3	
Yes	537	34.8	592	31.7	
Haemoglobin in Urine					0.4740
No	1496	97.0	1800	96.5	
Yes	47	3.0	65	3.5	
Leucocytes in Urine					0.1307
No	1309	84.8	1616	86.6	
Yes	234	15.2	249	13.4	
Protein in Urine					0.1276
No	1196	77.5	1404	75.3	
Yes	347	22.5	461	24.7	

Table 2. Baseline characteristics of women at T0 for numerical variables per study arm

	TBST		**Standard of care**	
	Mean	SD	Mean	SD
Altitude	1214.3	244.8	1393.4	225.7
Week of pregnancy at T0	24.7	1.9	24.6	2
Week of pregnancy at T1	34.7	1.8	34.6	1.9
Weight T0	51.8	7.2	51.9	6.5
Systolic blood pressure T0	103.9	10.1	104.1	9.8
Weight T1	54.1	7.4	54	6.7
Systolic blood pressure T1	104.5	9.6	104.2	8.7

Regarding children characteristics the distribution of participants by age, weight and height was similar between the standard of care and TBST groups (Table 3). The distribution of participants was also similar by sex, and maternal anaemia at baseline, iron supplement uptake and anti-malaria prophylaxis.

Differences were observed regarding the distribution of participants by region, urbanization, 1st pregnancy, pre-term birth and other characteristics. (Table 4)

Table 3. Baseline characteristics of children at T4 for numerical variables per study arm

	TBST		Standard of care	
	Mean	SD	Mean	SD
Age	24.9	1.04	24.8	0.86
Height	80.6	3.1	81	3.1
Weight	10.6	1.07	10.5	1.05

Table 4Baseline characteristics of children at T4 for categorical variables per study arm

	TBST		Standard of care		p-value
	n	%	n	%	
Region					<0.001
Amoron'iMania Region	509	41.6	627	38.3	
Bongolava	343	28.0	76	4.6	
Itasy	372	30.4	935	57.1	
Urban					<0.001
Rural	995	81.3	1,148	70.1	
Peri-Urban	229	18.7	490	29.9	
Sex					0.682
Male	607	49.6	825	50.4	
Female	617	50.4	813	49.6	
Pre-term birth					0.025
No	1,027	83.9	1,321	80.7	
Yes	197	16.1	317	19.4	
1 st pregnancy					0.011
No	797	65.1	1,140	69.6	
Yes	427	34.9	498	30.4	
Breastfeeding T4					<0.001
No	740	60.5	728	44.4	
Yes	484	39.5	910	55.6	
Maternal anaemia T0					0.509
No	634	51.8	828	50.6	
Yes	590	48.2	810	49.5	
Mother ever PZQ treatment			0		<0.001
No	1,066	88.1	1,492	92.4	
Yes	144	11.9	122	7.6	
Supplement: iron T0					0.186
No	228	20.0	273	17.9	
Yes	915	80.1	1,250	82.1	
Albendazole treatment T0					<0.001
No	714	58.3	1,182	72.7	
Yes	510	41.7	444	27.3	
Anti-malaria prophylaxis T0					0.882
No	858	70.1	1,144	69.8	

Yes	366	29.9	494	30.2	

Analysis for the primary outcomes

Maternal endpoint

Estimates of change in maternal Hb levels are reported in Table 3. In the intervention arm, the average change in Hb concentrations between T0 and T1 was 0.30 g/dL (95% CI: 0.14-0.47), while in the control arm 0.17 g/dL(0.05-0.29). No significant differences in Hb change were observed between arms(mean difference 0.11 g/dL (95% CI: -0.06, 0.28)). Estimates remain not statistically significant after adjustment for confounding (adjusted mean difference 0.08 g/dL (95% CI: -0.09, 0.26).

Table 5: Comparison of Hemoglobin level of between the enrollment and the 8th month of the pregnancy intervention vs. control arm

Characteristics	**Mean (SD) (T0)**	**Mean (SD) (T1)**	**Mean change in Hb (T1-T0) (95% CI)**	**Difference (95% CI)**	**Adjusted difference (95% CI)**
Hemoglobin level (g/dl) for TBST Group	11.3 (1.2)	11.5 (1.2)	0.30 (0.14-0.47)	0.11 (-0.06, 0.28)	0.08 (-0.09, 0.26)
Hemoglobin level (g/dl) for Standard of Care group	11.2 (1.3)	11.5 (1.2)	0.17(0.05-0.29)		

Sensitivity analysis performed restricting intervention arm to effectively treated showed similar results (Table 6).

Table 6. Comparison of Hemoglobin level of between the enrollment and the 8th month of the pregnancy: sensitivity analysis

Characteristics	**Mean (SD) (T0)**	**Mean (SD) (T1)**	**Mean change in Hb (T1-T0) (95% CI)**	**Difference (95% CI)**	**Adjusted difference (95% CI)**
Hemoglobin level (g/dl) for TBST Group	11.3 (1.2)	11.5 (1.2)	0.30 (0.13-0.46)	0.11 (-0.07, 0.28)	0.08 (-0.10, 0.25)
Hemoglobin level (g/dl) for Standard of Care group	11.2 (1.3)	11.5 (1.2)	0.17(0.05-0.29)		

Child endpoint

We estimated similar proportions of stunted children at T4 60.9% for the intervention and 59.6% for the control arms and observed no differences in the risk of stunting among treated participants compared to non-treated RR= 1.04 (0.79, 1.41). The differences remained non-significant after adjustment for the relevant covariates (aRR= 1.08 (0.82, 1.44)) (Table 7)

Table 7: Proportion of stunted children in the intervention and control arms

	n/N	p	95% CI	RR	aRR
Proportion of stunted children for TBST Group	746/1224	60.9%	(49.7 – 71.2)	1.04 (0.79, 1.41)	1.08 (0.82, 1.44)
Proportion of stunted children for Standard of Care group	976/1638	59.6%	(45.0 – 72.7)		

Analysis for the safety profile