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Mass Screening and Treatment on the Basis of Results of a *Plasmodium falciparum*-Specific Rapid Diagnostic Test Did Not Reduce Malaria Incidence in Zanzibar

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Abstract

Background.—Seasonal increases in malaria continue in hot spots in Zanzibar. Mass screening and treatment (MSAT) may help reduce the reservoir of infection; however, it is unclear whether rapid diagnostic tests (RDTs) detect a sufficient proportion of low-density infections to influence subsequent transmission.

Methods.—Two rounds of MSAT using *Plasmodium falciparum–specif*ic RDT were conducted in 5 hot spots (population, 12 000) in Zanzibar in 2012. In parallel, blood samples were collected

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on filter paper for polymerase chain reaction (PCR) analyses. Data on confirmed malarial parasite infections from health facilities in intervention and hot spot control areas were monitored as proxy for malaria transmission.

Results.—Approximately 64% of the population (7859) were screened at least once. *P. falciparum* prevalence, as measured by RDT, was 0.2% (95% confidence interval [CI], .1%–.3%) in both rounds, compared with PCR measured prevalences (for all species) of 2.5% (95% CI, 2.1%–2.9%) and 3.8% (95% CI, 3.2%–4.4%) in rounds 1 and 2, respectively. Two fifths (40%) of infections detected by PCR included non-falciparum species. Treatment of RDT-positive individuals (4% of the PCR-detected parasite carriers) did not reduce subsequent malaria incidence, compared with control areas.

Conclusions.—Highly sensitive point-of-care diagnostic tools for detection of all human malaria species are needed to make MSAT an effective strategy in settings where malaria elimination programs are in the pre-elimination phase.

Keywords

malaria elimination; mass screening and treatment; diagnostic tests; Zanzibar; subpatent; molecular methods; *Plasmodium falciparum* ; *Plasmodium vivax* ; *Plasmodium malariae* ; *Plasmodium ovale*

Malarial parasite infection among patients with fever has reduced significantly in Zanzibar over the past decade, from approximately 40% to <1%, following wide-scale deployment of multiple malaria control interventions [1-3]. Malaria transmission is now seasonal and focal, with 80% of the cases recorded in 2013 reported from 20% of public health facilities [3]. Such malaria foci are a well-documented phenomenon in low transmission areas [4-6] and can be targeted for transmission reduction strategies [7].

Zanzibar's primary surveillance system for malaria is the Malaria Early Epidemic Detection System (MEEDS), involving all public health facilities reporting (via mobile phone) weekly numbers of confirmed malaria cases (using rapid diagnostic tests [RDTs] or microscopy) among febrile patients tested [8]. This system detects symptomatic malarial parasite infections that involve relatively high parasite densities. Many studies have described asymptomatic low-density infections as a substantial reservoir of infection in settings of low and high transmission [9-13]. In Zanzibar, little is known about the magnitude of asymptomatic parasite carriers and their importance for sustained transmission.

Mass screening and treatment (MSAT) aims to detect and treat all malarial parasite infections, including asymptomatic parasite carriers, within the community [14]. The aim is to reduce the parasite reservoir (using multiple screening rounds) before the transmission season, with the intention of limiting onward transmission. The diagnostic tool usually used is an RDT, due to its ease of use and quick results. RDTs have well-documented limitations, including limited sensitivity for low-density falciparum (ie, <100 parasites/μL), and non-falciparum infections [15, 16]. Molecular methods, such as polymerase chain reaction (PCR), have demonstrated sensitivities as low as 0.02 parasites/μL [17], but currently there are few options for using this method for point-of-care diagnosis.

This study evaluated the impact of MSAT, using *P. falciparum*–specific RDT and artesunate-amodiaquine (ASAQ), on malaria incidence in Zanzibar. To determine the overall prevalence of malarial parasite infection, including low-density and non-falciparum infections not detected by the RDT, blood samples collected on filter paper underwent molecular analyses.

METHODS

Study Areas

Zanzibar is a semi-autonomous archipelago in Tanzania, located approximately 35 km from the coast of the mainland (population, approximately 1.3 million). Zanzibar is a malaria pre-elimination setting; transmission is low [18] and seasonal, with the majority of symptomatic cases recorded following the long rains, which occur from March to May.

MEEDS data from 2010 to 2011 were used to rank all public health facilities on Unguja island on the basis of malaria-positive test results and annual incidence of malaria infections per 1000 catchment population. The shehias (administrative units with populations up to 3000 people) served by the top-ranking health facilities were defined as hot spots. Twelve hot spot shehias were selected for the study. Five shehias (served by 3 public health facilities) were selected to be intervention shehias (4 from the central region [Jumbi, Tunguu, Bungi, and Ukongoroni] and 1 from the southern region [Mtende]; Figure 1). The remaining 7 shehias (served by 4 health facilities) were designated control shehias where MSAT was not performed. The intervention and control areas were chosen on the basis of similar transmission patterns, location, and population size.

When the study was designed, the estimated population of the intervention and control areas was approximately 10 000 each. However, the 2012 Tanzanian census estimated the population in the intervention area to be approximately 12 000.

MSAT Intervention

The intervention took place in May and June 2012 (weeks 20 and 24), before the onset of the expected seasonal increase of cases. Two rounds of household MSAT were conducted over 4 days each, with an interval of 4 weeks between rounds. The study aimed to include every household in the intervention shehias, but if the house was empty when visited, it was excluded for that round. Every person living permanently in the household was documented, even if not present, to estimate the percentage of the target population screened. Every household member was asked to provide a blood specimen by finger prick for an RDT and for dried blood spot analysis. The blood spot specimens were sent to Sweden for molecular analyses and stored at 4°C until use.

Malaria Indices

RDT—The RDT used for the study was Paracheck Pf(Orchid Biomedical Systems, India), in accordance with Zanzibar national guidelines at the time of the study. Paracheck Pf detects *P. falciparum*–specific histidine rich protein 2 (HRP-2). Participants with positive RDT results were provided treatment with ASAQ, as per national guidelines.

PCR—The prevalence of low-density and non-falciparum infections was assessed using PCR analysis of blood spot specimens (approximately 50 μL of blood; Whatman 3MM) from participants in the intervention sites. Molecular analyses took place in Sweden (Karolinska Institutet, Stockholm). The Chelex boiling method [19] was used to extract DNA from a 3-mm diameter punch from the blood spot specimens, and the cytochrome b (cytb) quantitative PCR (qPCR) with the SYBR Green real-time PCR assay, targeting the *Plasmodium* cytochrome b gene (Xu et al, unpublished data, 2014), was used for infection detection. DNA extraction was performed on pools of 6 samples [20] followed by duplicate cytb qPCR analysis, using 2 μL of extracted DNA as template. Each sample from *Plasmodium*-positive pools was individually extracted and subjected to cytb qPCR in duplicate, using 5 μL of extracted DNA as template. *Plasmodium*-positive specimens from this step underwent reextraction of DNA in duplicate and were subjected to cytb qPCR analysis in triplicate (using 5 μL DNA as template). The samples were defined as *Plasmodium* positive if results of at least one out of six of the final PCR analyses were positive. Positive and negative controls were incorporated into each PCR run. The detection limit for the cytb qPCR method for single DNA extraction is approximately 1–2 parasites/μL blood for *Plasmodium* species (Xu et al, unpublished data, 2014). Restriction fragment–length polymorphism analysis and sequencing of the cytb qPCR product was used for species determination.

An 18S qPCR method was used for parasite density quantification [21]. Briefly, samples with known densities of *Plasmodium* species were serially diluted, dropped on filter papers, air dried, and subjected to DNA extraction. 18S qPCR [21] was performed on the extracted DNA, and a standard curve was plotted using the quantification cycle (Cq) value and parasite density (parasites/μL). The mean Cq values from triplicate runs were calculated from the study samples.

Data Analysis

Data were double entered using CS Pro software. All analyses were done in Stata, version 12.1 (StataCorp, College Station, TX). Prevalence and 95% confidence intervals (CIs) and *P* values from χ^2 tests were calculated using binomial exact methods, adjusting for clustering within households and shehias. Differences between shehia, age group, and sex were investigated separately for each round of screening. For RDT sensitivity and specificity analyses, data from the 2 rounds were pooled, and the final PCR outcome for *P. falciparum* infections was defined as gold standard.

To assess subsequent transmission, malaria data were collected from 3 and 5 health facilities in the intervention and control areas, respectively. Diagnosis in the health facilities was made using *P. falciparum*–specific RDT or microscopy; only febrile patients were tested, according to national guidelines. Numbers of cases and the weekly and monthly incidence of malaria, defined as the number of cases per 1000 catchment facility population, were monitored until the end of 2012 and used as an indicator for transient fluctuations in transmission. Monthly incidences in the intervention and control sites following the survey were assessed using a generalized least squares random-effects model to account for differences between facilities.

Ethical Considerations

Before implementation, the study aims were described to the community leaders of each intervention area. During the screening, informed consent was obtained from each household head. The study was considered part of the Zanzibar Ministry of Health malaria surveillance strategy, and therefore it was deemed unnecessary to obtain ethical approval within Zanzibar. Ethical approval was granted through the Regional Ethics Review Board, Stockholm, Sweden (2013/836–32), and the Centers for Disease Control and Prevention determined that the study was a nonresearch activity.

RESULTS

MSAT Coverage

Overall, 86% of the census population in the intervention areas was documented by study enumerators (10 295 individuals). Approximately 17% (1786 individuals) of the enumerated population was absent for both rounds of screening. Of those present in each round, 4% (278 individuals) and 13% (723 individuals) refused to take part in the study in the first and second rounds, respectively. Based on census data, 53% (6389 individuals) and 43% (5046 individuals) of the target population was screened in rounds 1 and 2, respectively. In total, 76% of enumerated individuals (7859 of 10 295) were screened by RDT at least once, representing 64% of the population. The median age in both rounds was 19 years (range, 1 month–100 years). Overall, more females were tested than males (60% vs 40%). This difference was most pronounced between the ages of 15 and 50 years, in which over two thirds of people tested were female.

Malaria Indices

Prevalence of RDT-Detectable P. falciparum Infections—Twelve *P. falciparum* infections (0.2%; 95% CI, .1%–.3%) were detected using RDT in the first round. Ten of these were confirmed by PCR. RDT-detected infections were found in all shehias except Mtende (Table 1). Children aged 5–15 years had the highest RDT positivity rate (0.5%). No infections were detected in the oldest age group (50–100 years), and only 1 infection was found among children aged <5 years (Table 1).

In the second round, 8 *P. falciparum* infections (0.2%; 95% CI, .1%–0.3%) were detected using RDT. Four of these were confirmed by PCR. As in the first round, infections were detected in all shehias except Mtende. Two of the RDT-positive individuals in the second round had received treatment within the previous 2 weeks (both were PCR negative). All RDT-positive individuals were aged 8–28 years.

Prevalence of PCR-Detectable Malarial Parasite Infections—The prevalence of *P. falciparum* infections detected by PCR was significantly higher than the prevalence detected by RDT ($P < .001$), with values of 2.1% (95% CI, 1.7%–2.5%) and 2.6% (95% CI, 2.1%–3.1%) in the first and second rounds, respectively (Table 2). PCR identified 37 and 100 non-falciparum infections in rounds 1 and 2, respectively; 40% of these were mixed infections with *P. falciparum.* The PCR-based prevalence of *Plasmodium* was 2.5% (95%

CI, 2.1%–2.9%) and 3.8% (95% CI, 3.2%–4.4%; $P < .001$) in the first and second rounds, respectively.

Malarial parasite infections were detected by PCR in all shehias and in all age groups (Table 1). In the first round, children aged <5 years had the lowest prevalence (1.7%), with persons aged 15–25 years and those aged 25–50 years demonstrating the highest prevalence (2.9% and 3.7%, respectively; $P = .008$). In the second round, infections were evenly distributed over the age groups (Table 1).

Plasmodium Species Determination by PCR—The numbers of *Plasmodium* infections detected in each round are summarized in Table 2. Of the monoinfections detected by PCR, the majority were *P. falciparum* (83.0% in round 1 and 64.6% in round 2). *Plasmodium malariae* infection was the most common non-falciparum infection (14.9% and 26.2% of single infections in rounds 1 and 2, respectively), followed by *Plasmodium ovale* infection (1.4% and 7.7%, respectively). One *Plasmodium vivax* monoinfection was detected in the first round (the same individual tested negative for *P. vivax* in the second round) and 2 were detected in the second round. Four additional *P. vivax* infections were detected as part of a mixed infection in the second round (Table 2).

In the first round, 8.4% of the infections (13) were mixed *P. falciparum/P. malariae.* The prevalence of mixed infections increased to 29.3% (54) in the second round (Table 2). Mixed infections were only found in people aged 5–50 years in the first round ($P = .042$) but were evenly distributed across age groups in the second round ($P = .870$; Figure 2).

P. falciparum infections were distributed unevenly between shehias in both rounds ($P = .06$ in round 1 and $P < 0.001$ in round 2). Age was not associated with *P. falciparum, P. ovale*, or *P. vivax* infection, but in the first round people aged 15–25 years were more likely to be PCR positive for *P. malariae* ($P = .001$). This difference was not detected in the second round.

Parasite Density Determined by qPCR—qPCR was able to determine parasite density in 84% of samples (130) and 89% of samples (164) with positive 18S qPCR results in the first and second rounds, respectively (Table 3). The geometric mean parasite density was 4.1 parasites/μL (range, 0.1–16 130 parasites/μL) and 2.2 parasites/μL (range, 0.1–39 352 parasites/μL) in the first and second rounds, respectively. There was no association between parasite density and age ($P = .563$).

PCR Analysis of Paired Blood Samples From Rounds 1 and 2—Paired blood samples from the first and second rounds were available from 3268 participants (42% of total samples). Of these paired PCR results, 94% (3071) were negative and 0.2% (7) were positive in both rounds. Two percent (66) were positive in the first round but negative in the second round, whereas 4% (124) were negative in the first round but positive in the second round. Of the 66 who were positive in the first round but negative in the second, 90% had qPCR-determined parasite densities of <100 parasites/μL. Of the 124 who were negative in the first round but positive in the second round, only 2 had a parasite density of >100 parasites/μL. The parasite densities in the 7 matched positive samples were similar to those positive in only 1 round.

Sensitivity and Specificity of RDT for Detection of P. falciparum—Of 20 RDT-positive results, 14 (10 in the first round and 4 in the second round) were confirmed by PCR to be *P. falciparum* positive. This equates to treatment for 4% of people (14 of 332) with PCR-detectable infections. Three of 6 RDT-positive/PCR-negative individuals reported intake of antimalarial treatment in the previous 2 weeks. The RDT sensitivity and specificity, compared with qPCR, for *P. falciparum* detection was 5.6% (95% CI, 3.1%–9.2%) and 99.9% (95% CI, 99.8%–100.0%), respectively. However, the RDT sensitivity increased when the analysis was restricted to *P. falciparum* densities of >100 parasites/μL (47.6%; 95% CI, 25.7%–70.2%).

Passively Collected Health Facility Data on Confirmed Malarial Parasite Infections—Passive case detection using RDT/microscopy continued as usual in the public health facilities in the intervention and control areas. In the week following the second screening (week 25), the number of RDT/microscopy-positive cases recorded in the control area peaked at 10, with a peak of 7 recorded in the intervention area (Figure 3). In the 4 weeks following the second round of MSAT, 9 infections were detected at the Ukongoroni public health facility, contributing 50% of the total number of cases reported in all intervention health facilities during that time. The increase in cases was lower than that during the same period in the previous 2 years in intervention and control areas.

The average weekly incidence peaked at 1.6 and 0.6 cases per 1000 persons in the intervention and control areas in the weeks following the second round of screening (Figure 3). There was no difference in average monthly incidence between control and intervention areas at any point through to the end of 2012 ($P = .746$).

DISCUSSION

This study suggests that MSAT using *P. falciparum*–specific RDT as the diagnostic tool did not reduce subsequent malaria incidence, probably because only a minority (4% [14 of 332]) of the overall parasite carriers (as assessed by PCR) were detected and treated. More-sensitive point-of-care diagnostic tools are needed if MSAT interventions are going to be a useful strategy in pre-elimination settings [22]. A lack of impact following RDT-based MSAT has also been seen in Burkina Faso and Kenya [23, 24]. While these studies did not include a molecular diagnostic tool, it is possible that underlying subpatent infections, which were able to sustain transmission in the community, reduced the effects of the MSAT. An ideal solution would be a field-based PCR that can be used as a point-of-care diagnostic test [25, 26]. Field trials using a loop-mediated isothermal amplification kit (LAMP) [25, 27] have since been performed in Zanzibar.

While the low proportion of the parasite reservoir treated is probably the main reason there was no influence on subsequent incidence in our study, it is likely that the low population coverage also played a role. Absenteeism was a problem, particularly in the second round. School children and working adults are not necessarily present in their homes throughout the day, and in this study, owing to logistic reasons, it was not possible to follow up absent household members. One way of overcoming this problem would be to visit households in the evenings, when people are more likely to be present, or to visit schools to ensure all

school age children are screened [24]. Young men were particularly likely to be absent and were also most likely to refuse screening. In the first round of screening, males and people aged 15–25 years showed the highest infection rates, as has been reported in other settings of low endemicity [28, 29], and it is possible that screening with RDT would have been more effective if these risk groups had been better represented. A proportion of the absences may have been through community members actively choosing not to be available for screening, as the levels of absences and refusals were much higher in the second round. This highlights the importance of an effective community sensitization campaign before repeated screening rounds.

P. falciparum was the main species detected during the study. However, the proportion of non-falciparum infections detected in Zanzibar has been increasing in recent years. Two studies in 2013 found that 20% and 50% of infections detected using molecular methods were non-falciparum (unpublished data). In this study, the majority of the non-falciparum infections were of very low densities; however, detecting and clearing non-falciparum infections may become relatively more important as *P. falciparum* infections decrease further, as has been seen in other settings [30, 31]. Importantly, the presence of *P. vivax* has only been reported once previously in Zanzibar [32] and may pose a problem for elimination efforts.

Approximately 40% of the screened population was tested in both rounds, with many showing dichotomous PCR results. Several studies have demonstrated similar findings of fluctuations in parasite densities in settings of high [33, 34] and low [6] transmission. With such a high proportion of low-density parasitemias present in the study area, it is likely that many are on the cusp of detectability. However, importantly, the number of new infections detected in the second round highlights the importance of several screening rounds, even when sensitive diagnostic tests are used.

The second round had a higher prevalence of malarial parasite infections (3.8%), particularly non-falciparum infections. This increase may be related to rainfall that occurred between the 2 rounds, resulting in further spread of cases. However, it is not clear why this would particularly apply to non-falciparum infections. Temporal studies investigating transmission dynamics between *P. falciparum* and *P. malariae* or *P. ovale* are lacking. However, differential fluctuation in prevalence has been reported between *P. falciparum* and *P. vivax* in Cambodia [35], Vanuatu [36], and Peru [37]. In Vietnam, different vectors are thought to be responsible for the transmission of different species [38], although this has not been recorded in Zanzibar. The RDT now used in public health facilities in Zanzibar is SD Bioline (Standard Diagnostics, Republic of Korea), which is able to detect HRP-2 antigen specific to *P. falciparum* and pLDH specific to other *Plasmodium* species.

The study had several limitations, particularly related to the evaluation of subsequent transmission. Passive case detection is not an ideal proxy for onward transmission because it only detects symptomatic cases and is dependent on health facility utilization. A more effective measure of subsequent transmission would be to continue active case detection in the communities, using molecular methods, for several months after the intervention. In addition, while we have a reasonably accurate population measure through the census,

we cannot be certain that catchment areas assigned to facilities are accurate. While this may have affected the estimated incidence, the low numbers of cases treated during the intervention suggest that little impact would have been seen.

Bearing in mind the low sensitivity of current diagnostic tools available for the field, further understanding of the clustering of malaria cases and the possibility of delivering targeted presumptive treatment to those living near to RDT-positive cases or of implementing seasonal malaria mass drug administration in hot spots of transmission could represent pragmatic options to reduce the asymptomatic subpatent carriage of malarial parasites in Zanzibar, although the effectiveness of artemisinin-combination therapy on low-density infections will need to be considered [39, 40].

Malaria elimination was achieved in many parts of the world before molecular tools were available to detect low-density infections. The role that these infections play in transmission is still under debate [41], and it seems likely that they are less likely to transmit to mosquitoes than patent infections [42, 43]. However, in Zanzibar, where the mosquito vector is highly efficient, a high number of low-density infections is perhaps more likely to result in onward transmission, and therefore they are an important part of the parasite reservoir to target.

In conclusion, this study demonstrates that MSAT based on *P. falciparum*–specific RDT as a diagnostic tool did not reduce subsequent malaria incidence in Zanzibar, probably because of the large proportion of infections that were undetected by RDT and thus untreated but also because of the low population coverage. This highlights the importance of more-sensitive point-of-care diagnostic tools that can detect all human malaria species, if screen and treat programs are going to be a useful strategy in malaria preelimination settings.

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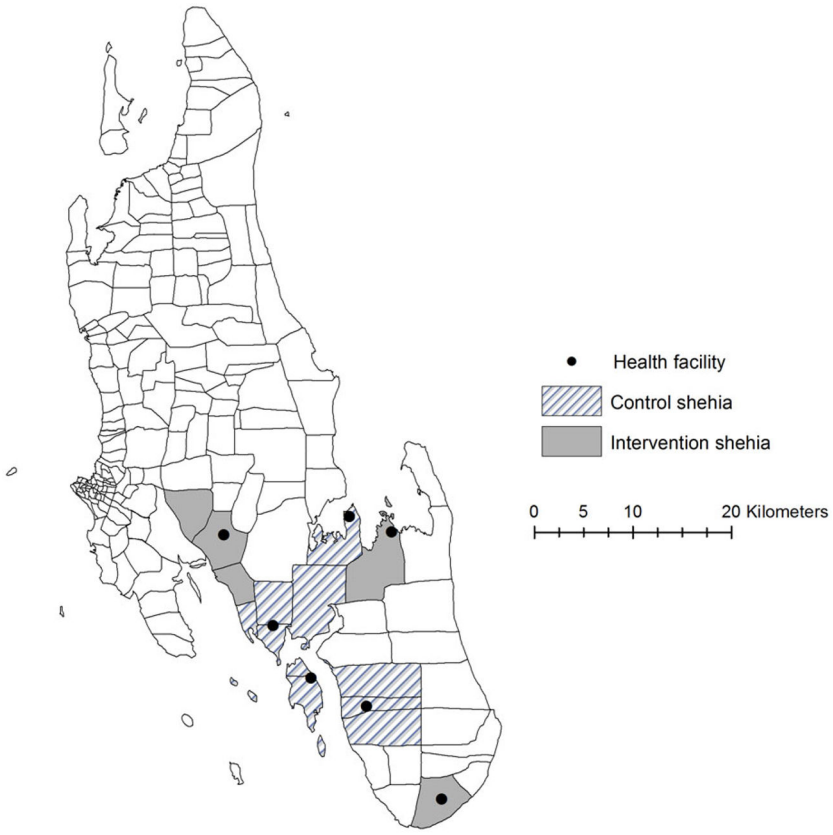

Figure 1.

Map of control (striped) and intervention (grey) study shehias and health facilities (black dot), Unguja Island, Zanzibar.

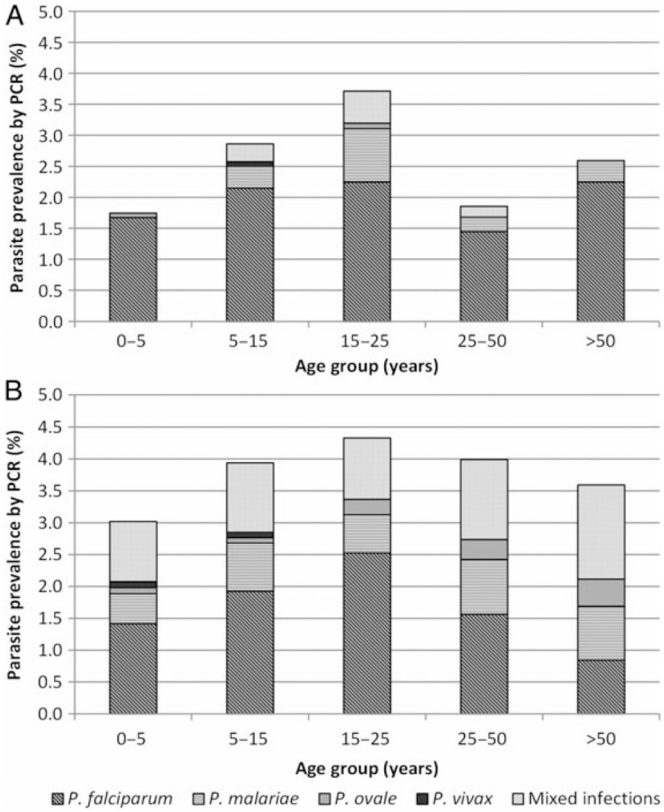

Figure 2.

Prevalence of *Plasmodium* species, by polymerase chain reaction (PCR) findings, across age groups in round 1 (*A*) and round 2 (*B*).

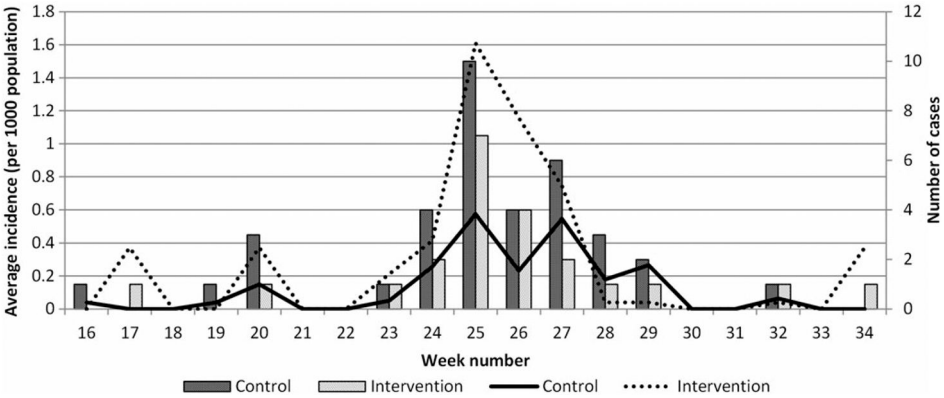

Figure 3.

Number of confirmed malarial parasite infections reported from public health facilities (bars) and average weekly incidence in the control and intervention areas (lines). Screening occurred in weeks 20 and 24.

Table 1.

Malarial Parasite Infection Prevalence and Distribution Across Age Group, Sex, and Geographical Distribution, by Rapid Diagnostic Testing (RDT) and Polymerase Chain Reaction (PCR) Analysis, in Rounds 1 and 2 of Mass Screening and Treatment, Unguja Island, Zanzibar

	RDT-Based Prevalence, No. (%)		PCR-Based Prevalence, No. (%)	
	Round 1	Round 2	Round 1	Round 2
Variable	**(n = 6381)**	**(n = 5046)**	**(n = 6233)**	**(n = 4894)**
Overall	12 (0.2)	8 (0.2)	154 (2.5)	185 (3.8)
Age, y				
0–5	1 (0.1)	3 (0.3)	24 (1.7)	32 (3.0)
5–15	7 (0.5)	2 (0.2)	40 (2.9)	48 (4.0)
15–25	2 (0.2)	1 (0.1)	43 (3.7)	36 (4.3)
25–50	2 (0.1)	2 (0.3)	32 (1.9)	51 (4.0)
50–100	0 (0.0)	0 (0.0)	15 (2.6)	17 (3.6)
Sex				
Female	8 (0.2)	3 (0.1)	77 (2.1)	108 (3.7)
Male	4 (0.2)	5 (0.3)	77 (2.9)	77 (4.0)
Location				
Bungi	1 (0.1)	3 (0.3)	35 (2.7)	35 (3.8)
Tunguu	5 (0.4)	2 (0.2)	32 (2.4)	39 (3.8)
Jumbi	4 (0.2)	1 (0.1)	53 (2.9)	53 (3.6)
Ukongoroni	2 (0.3)	2 (0.3)	8 (1.1)	36 (5.8)
Mtende	0 (0.0)	0 (0.0)	26 (2.5)	22 (2.6)

Table 2.

Plasmodium Species Detected by Polymerase Chain Reaction Analysis Among Malarial Parasite–Positive Specimens in Rounds 1 and 2, Unguja Island, Zanzibar

Species	Round 1, No. (%) (n = 154)	Round 2, No. (%) (n = 185)
P. falciparum	117 (76.0)	84 (45.4)
P. falciparum, P. malariae	13 (8.4)	27 (14.6)
P. falciparum, P. malariae, P. ovale	0	5 (2.7)
P. falciparum, P. ovale	0	9 (4.9)
P. falciparum, P. vivax	0	1 (0.5)
P. malariae	21 (13.6)	35 (18.9)
P. malariae, P. ovale	0	9 (4.9)
P. malariae, P. ovale, P. vivax	0	2 (1.1)
P. malariae, P. vivax	0	1 (0.5)
P. ovale	2 (1.3)	10 (5.4)
P. vivax	1 (0.6)	2 (1.1)

Table 3.

Parasite Density Determined by Quantitative Polymerase Chain Reaction Analysis in Rounds 1 and Round 2, Unguja Island, Zanzibar

Parasites/μL of Blood	Round 1	Round 2
Not quantifiable	24 (15.6)	21 (11.4)
0.1–10	88 (57.1)	127 (68.7)
10–50	20 (13.0)	28 (15.1)
50–100	7 (4.6)	1 (0.5)
100–500	9 (5.8)	3 (1.6)
500–5000	6 (3.9)	5 (2.7)
Total	154	185

Data are no. or no. (%) of samples