Annual Versus Biannual Mass Azithromycin Distribution and Malaria Parasitemia During the Peak Transmission Season Among Children in Niger

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Conflicts of Interest. None to report.

ABSTRACT

Introduction. Azithromycin has modest efficacy against malaria, and previous cluster randomized trials have suggested that mass azithromycin distribution for trachoma control may play a role in malaria control. We evaluated the effect of annual versus biannual mass azithromycin distribution over a three-year period on malaria prevalence during the peak transmission season in a region with seasonal malaria transmission in Niger.

Methods. Twenty-four communities in Matamèye, Niger were randomized to annual mass azithromycin distribution (3 distributions to the entire community during the peak transmission season) or biannual targeted azithromycin distribution (6 distributions to children <12 years, including 3 in the peak transmission season and 3 in the low transmission season). Malaria indices were evaluated at 36 months during the high transmission season.

Results. Parasitemia prevalence was 42.6% (95% confidence interval [CI] 31.7 to 53.6%) in the biannual distribution arm compared with 50.6% (95% CI 40.3 to 60.8%) in the annual distribution arm (*P*=0.29). There was no difference in parasite density or hemoglobin concentration in the two treatment arms.

Conclusions. Additional rounds of mass azithromycin distribution during low transmission may not have a significant impact on malaria parasitemia measured during the peak transmission season.

Trial Registration. clinicaltrials.gov NCT00792922

INTRODUCTION

Studies of mass azithromycin for trachoma control have suggested that azithromycin may play a role in malaria control.[1,2] Azithromycin has activity against the apicoplast, an organelle that is required for plasmodium survival.[3-6] Although it has been shown to be inferior to other antimalarial treatment options as first-line treatment, azithromycin is known to be safe for mass distribution, with millions of doses distributed annually as part of trachoma control programs.[7,8] Mass azithromycin distribution for trachoma control has been shown to significantly decrease child mortality, an effect which may be partially mediated by reduction in infection burden.[9] Azithromycin has also been safely and effectively used as part of combination treatment strategies for the prevention of malaria in pregnant women.[10] Even though azithromycin monotherapy is not recommended for the treatment of malaria at the individual level, mass azithromycin distribution in malaria endemic regions could be beneficial if it reduces the malaria burden in communities.

Two previous substudies of a randomized controlled trial of mass azithromycin distribution for trachoma control in Niger found conflicting results comparing annual and biannual mass azithromycin for malaria.[1,11] Compared with a single mass azithromycin distribution, two mass azithromycin distributions (6 months apart) was shown to lead to a significant reduction in malaria parasitemia.[1] However, there was no significant difference in malaria prevalence or parasitemia during the high transmission season between three annual compared with six biannual mass azithromycin distributions in a different set of communities.[11] Here, we report 36-month malaria parasitemia outcomes from the same trial collected during the peak transmission season after 3 annual or 6 biannual azithromycin distributions in the same communities in which a decrease in parasitemia prevalence was previously noted during the low

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transmission season following two mass azithromycin distributions compared with a single mass azithromycin distribution.

METHODS

Study design. The Partnership for the Rapid Elimination of Trachoma (PRET) was a consortium of community-randomized trials conducted in Niger, The Gambia, and Tanzania (clinicaltrials.gov NCT00792922) comparing mass azithromycin distribution strategies for trachoma control. Complete methods for the PRET study have been reported previously.[12,13] The present report focuses solely on the Niger trial, which enrolled participants in Matamèye District, Zinder Region between May 2010 and August 2013. Communities were randomized to one of four arms in a 2x2 factorial design: 1) annual treatment of all individuals in the community with a treatment coverage target of 80%; 2) annual treatment of all individuals in the community with a treatment coverage target of >90%; 3) biannual treatment of children aged 12 and under with a treatment coverage target of 80%; or 4) biannual treatment of children aged 12 and under with a treatment coverage target of >90%. Here, we report outcomes comparing annual versus biannual distribution with treatment coverage targets of 80% (the standard WHO treatment coverage target for trachoma control). Communities were eligible for enrollment in the trial if they had a population between 250 and 600 at the most recent government census and clinical trachoma prevalence of at least 10% at the time of this census. Twenty-four grappes (henceforth, "community") from 6 Centres de Santé Intégrées (CSI; larger government health unit) are included in this analysis.

Ethical approval was obtained from the Committee on Human Research at the University of California, San Francisco and the Comité d'Ethique du Niger (the Ethical Committee of Niger). Given low literacy rates in the study area, both institutional review boards approved

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verbal informed consent from local chiefs of each study community prior to randomization, and verbal informed consent from the parent or guardian of each participant.

Study Setting. This study was conducted in Matemèye District, Zinder Region, Niger, which is situated in the Sahel of sub-Saharan Africa and has a highly seasonal malaria epidemic.[14,15] The region is holoendemic for malaria, and cases peak shortly after the peak rainfall, typically in September.[15] At the time of the study, the only programmatic activity ongoing in the region for malaria control was bednet distribution. There was no seasonal malaria chemoprevention program in this region at the time of the study. Individuals who self-referred for treatment to their local health post were treated or referred elsewhere for treatment.

Randomization. Communities were randomized by stratified block randomization within each CSI by high or low clinical trachoma prevalence in the community. Communities were categorized as high or low trachoma prevalence based on whether they were above or below the median trachoma prevalence in a given CSI. The random allocation sequence was generated by TCP using the statistical package R (version 2.12; R Foundation for Statistical Computing, Vienna, Austria; www.r-project.org).

Intervention. In the annual mass azithromycin distribution arm, all individuals aged 6 months and older in all communities were offered one mass azithromycin distribution per year. In the biannual mass azithromycin distribution arm, children aged 6 months to 12 years in all communities were offered two mass azithromycin distributions at approximately 6 month intervals: once during the low and once during the high transmission season. Enrolled children under 6 months of age in both groups and those allergic to macrolides were offered topical tetracycline ointment (1%) to be applied to both eyes (unobserved) twice a day for six weeks.

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Target antibiotic coverage in both arms was 80%, the WHO standard for trachoma control programs.

Clinical and laboratory assessments. Blood samples for determination of malaria prevalence were collected from a random sample of children in both arms at 36 months after baseline. Children were randomly selected from an annual census that was conducted prior to the annual treatment in each community. Blood samples were collected in September 2013, during the high transmission season for malaria.[15,16] A target sample of 50 children randomly selected from each village was used for collection of blood samples for thick smear and hemoglobin assessment. In communities with fewer than 50 children, blood samples were collected from all children in the community. Thick blood smears were collected on glass slides, air-dried, and stored at room temperature. Each thick blood smear was stained and examined by two microscopists masked to each other at Zinder Regional Hospital in Niger with 3% Giemsa who determined the presence or absence of *Plasmodium* parasites on the slides. Each microscopist independently counted the number of asexual parasites per 200 white blood cells to assess parasite density, and the median of these two parasite density readings was used in analysis. Hemoglobin concentration was determined using HemoCue AB (Ängelholm, Sweden).

Sample size. The sample size for the trial was based on the primary trachoma outcome.[13] For the malaria outcome, the power calculation was based on malaria estimates from the low transmission season.[14] We estimated that a random sample of 50 children in 12 communities per arm (24 communities total) would provide 80% power to detect a 3% difference in malaria parasitemia, assuming a baseline prevalence of 10% and an intraclass correlation coefficient of 0.075.

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Statistical analysis. All analyses were conducted as intention-to-treat. To compare malaria prevalence between annual and biannual mass azithromycin distribution arms, an unadjusted mixed effects logistic regression model was used with a fixed effect for study arm and a random effect for community to account for clustering within communities. Parasitemia and hemoglobin levels were compared using a linear mixed effects regression model with a fixed effect for study arm and a random effect for community. As a sensitivity analysis, we repeated analyses using the community-level prevalence of malaria metrics, which does not require assumptions related to the structure of the covariance matrix. All *P*-values were calculated with an exact permutation test accounting for the stratified randomization scheme. All analyses were conducted in R (version 3.3.1, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Of 1,037 children from 24 communities with blood samples for malaria, 511 (49.3%) were in the annual mass azithromycin distribution arm and 526 (50.7%) were in the biannual mass azithromycin distribution arm (Figure 1). Baseline characteristics between children in communities randomized to annual and biannual distribution were well balanced (Table 1). Antibiotic coverage among children 6-60 months of age was 88.8% in the annual distribution arm and 80.8% in the biannual distribution arm (Table 2). Antibiotic coverage was similar at each mass azithromycin distribution time point between the two study arms (Table 2).

Malariometric analyses are presented in Table 3. Malaria prevalence in the biannual distribution arm was lower than in the annual distribution arm (42.6% versus 50.6%), but this difference was not statistically significant in a model accounting for clustering (*P*=0.29). The ICC for malaria parasitemia was 0.10 (95% CI 0.05 to 0.19). We did not detect a difference between study arms in mean parasite density (mean parasite density 6,260 in the annual arm

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versus 10,660 in the biannual arm, *P*=0.57). Clinical malaria prevalence, defined as the presence of parasitemia plus fever, was similar in the biannual and annual arms (5.9% versus 6.8%, *P*=0.69). There was no difference in hemoglobin between the annual and biannual arms (9.2 g/dL in the annual compared to 9.5 g/dL in the biannual arm, *P*=0.21). Results were robust to analyses at both the community and individual levels (Table 3).

DISCUSSION

After 36 months of biannual mass azithromycin distribution, there was a non-significant decrease in malaria parasitemia relative to annual mass azithromycin distribution. In the same communities approximately 2 years prior, malaria parasitemia prevalence during the low transmission season was significantly lower in communities that had received two mass azithromycin distributions compared with those that had received a single mass azithromycin distribution (19.5% compared to 29.8%).[1] These results are consistent with mathematical models, which have suggested that mass drug administration for malaria may be maximally effective during the low transmission season.[17-19] It is possible that azithromycin is more effective at the community level for malaria control during the low transmission season because, the probability of reinfection is lower after treatment. A previous study showed a short-term reduction (73%) in malaria parasitemia prevalence in communities with low (6% at baseline) prevalence following mass azithromycin distribution, but no long-term effect of azithromycin distribution at the community level.[20] A previous analysis of the PRET Niger study demonstrated a non-significant reduction in all-cause mortality with biannual compared to annual azithromycin distribution over the 36-month period (O'Brien et al, under review). Given that azithromycin has only modest efficacy against *P. falciparum*[21,22], the major cause of malaria in Niger, azithromycin may offer incomplete protection against clinical malaria. Azithromycin may have a

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greater impact on malaria control during the low transmission season compared to the high transmission season.

The results of this study are consistent with previously-reported results from 24 different communities randomized to annual versus biannual mass azithromycin distribution over a 36-month period in the same study.[11] In these studies, samples were collected during the high transmission season. It is possible that multiple mass azithromycin distributions increase selection of azithromycin-resistant strains, although we were unable to assess this here. Previous studies have demonstrated mixed results for the effect of azithromycin treatment on the development of plasmodium resistance.[2,23] Testing azithromycin resistance in plasmodium is challenging, and the degree to which resistance could mitigate the protective effects of azithromycin is unknown. As tests for azithromycin-resistant malaria become more readily available, it will be important to assess the role of azithromycin resistance in malaria in the context of mass drug administration.

The results of this analysis must be considered in the context of several limitations. In the annual distribution arm, all individuals in the community were eligible for treatment, whereas treatments were targeted to children 12 years of age and under in the biannual arm. It is possible that treatment of additional individuals in the community outside of the targeted age range had an effect on malaria transmission. Thus, any effect of intervention cannot be attributed solely to treatment administration frequency. The WHO coverage target for trachoma control is 80%. Not every individual in every community was treated at each time point, and treatment coverage tended to be lower for older age groups. Blood samples for malaria testing were only collected at 12 and 36 months after randomization. We were unable to assess baseline malaria prevalence. In addition, baseline data on bed net coverage or use were not available. These data were collected

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in very different malaria transmission seasons, so we are unable to measure whether the burden of malaria was decreasing over time during ongoing mass azithromycin distribution. As expected, malaria parasitemia prevalence was higher in the present report than was noted in the same communities previously, likely due to different seasons of data collection. Given that all communities received either annual or biannual mass azithromycin distribution and there was no untreated comparison group, it may be difficult to detect modest differences in malaria parasitemia between communities treated with different frequencies over a three-year period. Studies with a comparison group that did not receive azithromycin may be required to fully understand the effects of mass azithromycin distribution for malaria control. Larger studies may be necessary to detect the effect of azithromycin on malaria parasitemia.

Biannual mass azithromycin distribution during a three-year period led to a non-statistically significant decrease in malaria parasitemia during the peak transmission season relative to annual mass azithromycin distribution. Although azithromycin has activity against malaria during high transmission, any protective effect may be mitigated by high rates of reinfection. In trachoma-endemic regions, there may be some collateral benefit of mass azithromycin distribution on malaria parasitemia, although more frequent dosing in combination with other antimalarials may be required for azithromycin to contribute significantly to malaria control in regions with seasonal malaria transmission.

REFERENCES

1 Gaynor BD, Amza A, Kadri B, *et al.* Impact of mass azithromycin distribution on malaria parasitemia during the low-transmission season in Niger: a cluster-randomized trial. *Am J Trop Med Hyg* 2014; **90**: 846–51.

2 Sadiq ST, Glasgow KW, Drakeley CJ, *et al.* Effects of azithromycin on malariometric indices in The Gambia. *The Lancet* 1995; **346**: 881–2.

3 Rosenthal PJ. Azithromycin for Malaria? *Am J Trop Med Hyg* 2016; **95**: 2–4.

4 Sidhu ABS, Sun Q, Nkrumah LJ, Dunne MW, Sacchettini JC, Fidock DA. In Vitro Efficacy, Resistance Selection, and Structural Modeling Studies Implicate the Malarial Parasite Apicoplast as the Target of Azithromycin. *The Journal of Biological Chemistry* 2007; **282**: 2494–504.

5 Dahl EL, Rosenthal PJ. Multiple Antibiotics Exert Delayed Effects against the Plasmodium falciparum Apicoplast. *Antimicrob Agents Chemother* 2007; **51**: 3485–90.

6 Gaillard T, Dormoi J, Madamet M, Pradines B. Macrolides and associated antibiotics based on similar mechanism of action like lincosamides in malaria. *Malaria Journal* 2016; : 1–11.

7 Taylor HR, Burton MJ, Haddad D, West F, Wright H. Trachoma. *The Lancet* 2014; **384**: 2142–52.

8 Melese M, Alemayehu W, Lakew T, *et al.* Comparison of Annual and Biannual Mass Antibiotic Administration for Elimination of Infectious Trachoma. *JAMA* 2008; **299**: 778–84.

9 Porco TC, Gebre T, Ayele B, *et al.* Effect of Mass Distribution of Azithromycin for Trachoma Control on Overall Mortality in Ethiopian Children: A Randomized Trial. *JAMA* 2009; **302**: 962–8.

10   Unger HW. Sulphadoxine-pyrimethamine plus azithromycin for the prevention of low birthweight in Papua New Guinea: a randomised controlled trial. 2015; : 1–16.

11   OBrien KS, Cotter SY, Amza A, *et al.* Mass azithromycin and malaria parasitemia in Niger: Results from a community-randomized trial. *Am J Trop Med Hyg* 2016; **In press**: 1–7.

12   Stare D, Harding-Esch E, Munoz B, *et al.* Design and Baseline Data of a Randomized Trial to Evaluate Coverage and Frequency of Mass Treatment with Azithromycin: The Partnership for Rapid Elimination of Trachoma (PRET) in Tanzania and The Gambia. *Ophthalmic Epidemiology* 2011; **18**: 20–9.

13   Amza A, Kadri B, Nassirou B, *et al.* A cluster-randomized trial to assess the efficacy of targeting trachoma treatment to children. *Clin Infect Dis* 2016; **December 12, 2016**.

14   Doudou MH, Mahamadou A, Ouba I, *et al.* A refined estimate of the malaria burden in Niger. *Malaria Journal* 2012; **11**: 89.

15   Guillebaud J, Mahamadou A, Zamanka H, *et al.* Epidemiology of malaria in an area of seasonal transmission in Niger and implications for the design of a seasonal malaria chemoprevention strategy. *Malaria Journal* 2013; **12**: 379.

16   Burki TK. Malaria and malnutrition: Niger's twin crises. *The Lancet* 2013; **382**: 587–8.

17   Gao D, Amza A, Nassirou B, *et al.* Optimal Seasonal Timing of Oral Azithromycin for Malaria. *American Journal of Tropical Medicine and Hygiene* 2014; **91**: 936–42.

18   Gu W, Killeen GF, Mbogo CM, Regens JL, Githure JI, Beier J. An individual-based model of Plasmodium falciparum malaria transmission on the coast of Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2003; **97**: 43–50.

19   Okell LC, Griffin JT, Kleinschmidt I, *et al.* The Potential Contribution of Mass Treatment to the Control of Plasmodium falciparum Malaria. *PLoS ONE* 2011; **6**: e20179–11.

20   Schachterle SE, Mtove G, Levens JP, *et al.* Short-Term Malaria Reduction by Single-Dose

       Azithromycin during Mass Drug Administration for Trachoma, Tanzania. *Emerg Infect Dis*

       2014; **20**: 1–9.

21   Anderson SL, Oloo AJ, Gordon DM, *et al.* Successful double-blinded, randomized,

       placebo-controlled field trial of azithromycin and doxycycline as prophylaxis for malaria in

       western Kenya. *clinical infectious diseases* 1998; **26**: 146–50.

22   Taylor WR, Richie TL, Fryauff DJ, *et al.* Malaria Prophylaxis Using Azithromycin: A

       Double-Blind, Placebo-Controlled Trial in Irian Jaya, Indonesia. *clinical infectious diseases*

       1999; **28**: 74–81.

23   Sykes A, Hendriksen I, Mtove G, *et al.* Azithromycin plus Artesunate versus Artemether-

       Lumefantrine for Treatment of Uncomplicated Malaria in Tanzanian Children: A

       Randomized, Controlled Trial. *clinical infectious diseases* 2009; **49**: 1195–201.

FIGURE LEGENDS

Figure 1. CONSORT flow diagram

Figure 2. Map of study communities. Communities in the annual distribution arm are in red and biannual distribution in blue. Larger circles represent larger total population size.

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Table 1. Baseline characteristics by randomization arm

	Annual Distribution N=12	**Biannual Distribution** N=12
No. children age 6-60 months per community, mean (range)	141 (44 to 580)	136 (59 to 224)
Proportion female (95% CI)	51.9% (50.9 to 52.8%)	51.6% (50.1 to 53.1%)
Prevalence of TF (95% CI)	27.0% (16.0 to 38.0%)	24.7% (16.4 to 32.9%)
Prevalence of TI (95% CI)	8.7% (4.6 to 12.8%)	9.1% (5.0 to 13.3%)

Abbreviations: No.: number; 95% CI: 95% confidence interval; TF: trachomatous inflammation – follicular; TI: trachomatous inflammation – intense

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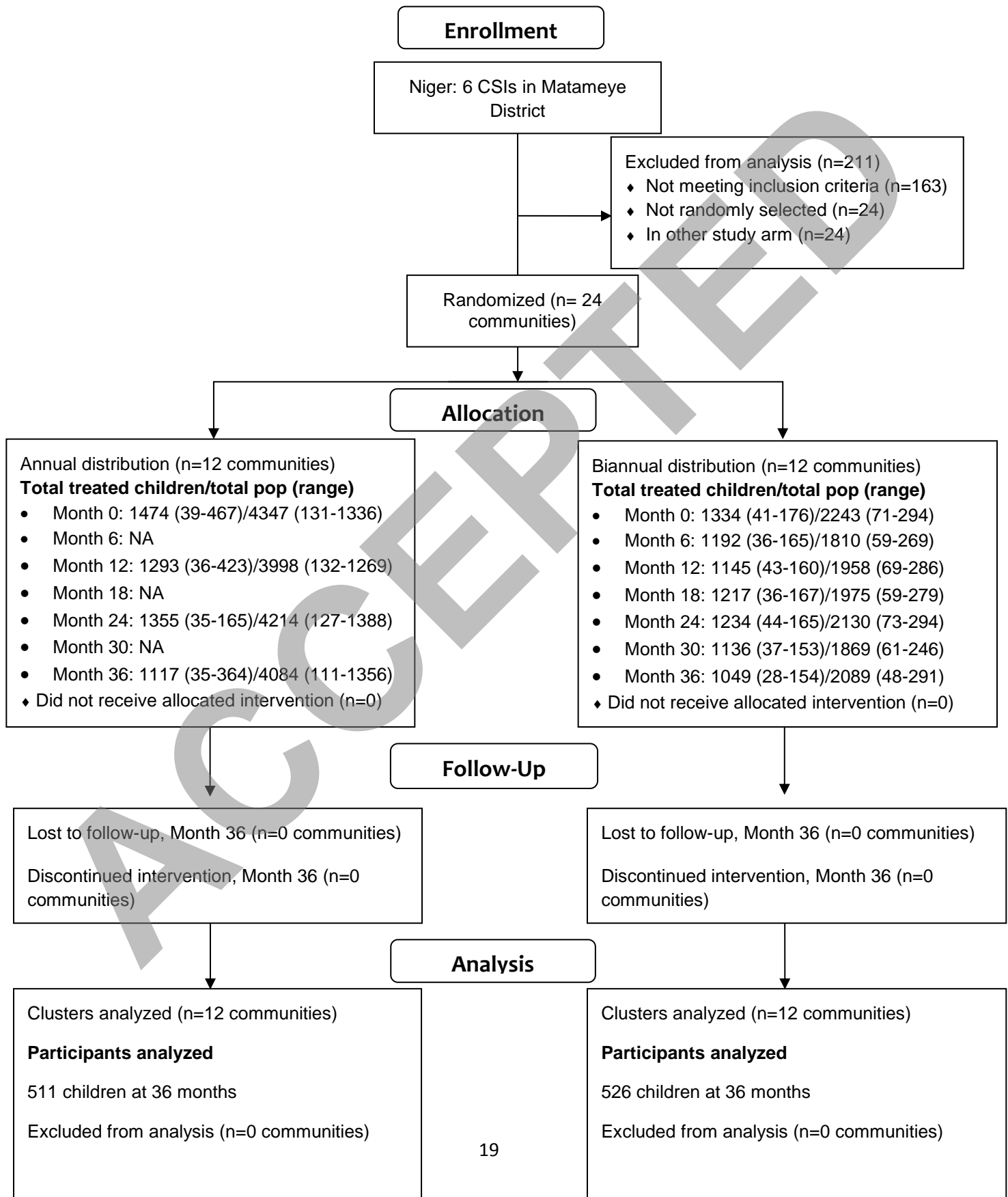**Table 2.** Average antibiotic coverage at each mass azithromycin distribution by randomization arm by age group

	6-60 months		5-12 years		>12 years	
	Annual Distribution (95% CI)	**Biannual Distribution (95% CI)**	**Annual Distribution (95% CI)**	**Biannual Distribution (95% CI)**	**Annual Distribution (95% CI)**	**Biannual Distribution (95% CI)**
Month 0	88.8% (86.1%- 91.5%)	80.8% (76.4%-85.1%)	79.4% (76.2%-82.4%)	71.2% (66.8%-75.2%)	65.8% (62.8%-68.7%)	0.7% (0.3%-1.3%)
Month 6	--	78.6% (74.6%-82.6%)	--	57.2% (54.8%-59.4%)	--	--
Month 12	85.7% (83.2%-88.1%)	81.1% (78.3%-84.0%)	73.2% (69.1%-76.3%)	65.2% (60.1%-69.9%)	62.8% (60.2%-65.5%)	0.3% (0.0%-0.8%)
Month 18	--	82.2% (77.2%-87.1%)	--	63.4% (59.2%-67.1%)	--	--
Month 24	87.2% (84.9%-89.6%)	84.3% (80.1%-88.6%)	75.9% (69.8%-81.4%)	65.4% (59.7%-69.5%)	62.2% (58.8%-65.4%)	0.2% (0.1%-0.4%)
Month 30	--	78.2% (74.0%-82.4%)	--	57.1% (52.9%-62.3%)	--	--
Month 36	79.7% (75.4%-84.0%)	75.8% (71.6%-80.1%)	71.3% (67.7%-73.6%)	61.3% (54.9%-66.6%)	66.8% (59.0%-75.0%)	4.8% (0.2%-15.8%)

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Table 3. Malariometric outcomes among children aged 6-60 months by randomization arm

	Annual Distribution	**Biannual Distribution**	*P-value*[1]	**Mean Difference (95% CI)**[2]	*P-value*[2]
Parasitemia prevalence (95% CI)	50.6% (40.3 to 60.8%)	42.6% (31.7 to 53.6%)	0.29	-7.9% (-22.0 to 6.2%)	0.28
Parasite density, mean (95% CI)	6,260 (3,730 to 8,800)	10,660 (6,370 to 14,950)	0.57	4,985 (-1,309 to 11,229)	0.41
Clinical malaria[3] prevalence (95% CI)	6.8% (4.8 to 9.4%)	5.9% (4.0 to 8.3%)	0.69	-0.3% (-4.4 to 3.9%)	0.89
Hemoglobin, g/dL, mean (95% CI)	9.2 (9.1 to 9.4)	9.5 (9.3 to 9.6)	0.21	0.2 (-0.2 to 0.5)	0.70

[1]Calculated with an exact permutation test in a mixed effects model with a fixed effect for study arm and random effect for community; [2]Mean difference in each measure at the community level; [3]Defined as malaria parasitemia and fever (temperature ≥38.5°C)

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Figure 1. CONSORT Diagram

Figure 2