ORIGINAL ARTICLE

Trial of Three Rounds of Mass Azithromycin Administration for Yaws Eradication

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ABSTRACT

BACKGROUND

Treponema pallidum subspecies *pertenue* causes yaws. Strategies to better control, eliminate, and eradicate yaws are needed.

METHODS

In an open-label, cluster-randomized, community-based trial conducted in a yaws-endemic area of Papua New Guinea, we randomly assigned 38 wards (i.e., clusters) to receive one round of mass administration of azithromycin followed by two rounds of target treatment of active cases (control group) or three rounds of mass administration of azithromycin (experimental group); round 1 was administered at baseline, round 2 at 6 months, and round 3 at 12 months. The coprimary end points were the prevalence of active cases of yaws, confirmed by polymerase-chain-reaction assay, in the entire trial population and the prevalence of latent yaws, confirmed by serologic testing, in a subgroup of asymptomatic children 1 to 15 years of age; prevalences were measured at 18 months, and the between-group differences were calculated.

RESULTS

Of the 38 wards, 19 were randomly assigned to the control group (30,438 persons) and 19 to the experimental group (26,238 persons). A total of 24,848 doses of azithromycin were administered in the control group (22,033 were given to the participants at round 1 and 207 and 2608 were given to the participants with yaws-like lesions and their contacts, respectively, at rounds 2 and 3 [combined]), and 59,852 doses were administered in the experimental group. At 18 months, the prevalence of active yaws had decreased from 0.46% (102 of 22,033 persons) at baseline to 0.16% (47 of 29,954 persons) in the control group and from 0.43% (87 of 20,331 persons) at baseline to 0.04% (10 of 25,987 persons) in the experimental group (relative risk adjusted for clustering, 4.08; 95% confidence interval [CI], 1.90 to 8.76). The prevalence of other infectious ulcers decreased to a similar extent in the two treatment groups. The prevalence of latent yaws at 18 months was 6.54% (95% CI, 5.00 to 8.08) among 994 children in the control group and 3.28% (95% CI, 2.14 to 4.42) among 945 children in the experimental group (relative risk adjusted for clustering and age, 2.03; 95% CI, 1.12 to 3.70). Three cases of yaws with resistance to macrolides were found in the experimental group.

CONCLUSIONS

The reduction in the community prevalence of yaws was greater with three rounds of mass administration of azithromycin at 6-month intervals than with one round of mass administration of azithromycin followed by two rounds of targeted treatment. Monitoring for the emergence and spread of antimicrobial resistance is needed. (Funded by Fundació "la Caixa" and others; ClinicalTrials.gov number, NCT03490123.)

YAWS, AN INFECTION CAUSED BY *TREPONEMA pallidum* subspecies *pertenue*, affects the skin and long bones of children in poor rural communities in the tropics.[1] It was estimated that in the absence of an eradication campaign, yaws would cause 1.6 million disability-adjusted life-years lost between 2015 and 2050.[2]

The finding that a single oral dose of azithromycin is as effective as penicillin G benzathine in the treatment of the disease[3] prompted the World Health Organization (WHO) to launch a worldwide program to eradicate yaws by 2030.[4,5] Eradication refers to the complete and permanent reduction in the number of new cases to zero, whereas elimination refers to such reduction in a certain geographic area. The current principle of yaws eradication (the Morges strategy) is based on a single round of mass administration of azithromycin in the entire community, followed by targeted treatment of active cases and their contacts every 6 months.[4,5] As of August 2021, the WHO, in collaboration with a Brazilian pharmaceutical company, has provided more than 10 million tablets to support mass drug administration and surveillance activities in several countries, including Cameroon, the Central African Republic, the Democratic Republic of Congo, Ivory Coast, Ghana, Liberia, Papua New Guinea, the Solomon Islands, and Vanuatu.[6]

Studies conducted in Papua New Guinea, the Solomon Islands, and Ghana have all shown that one round of mass drug administration will not suffice to stop transmission of infection,[7-9] presumably because of the relapse of untreated latent infections among persons who had not been treated during the mass drug administration campaign.[10] Mathematical modeling data show that during treatment campaigns, a high coverage of latent cases is required in order to have a high likelihood of achieving eradication.[11] However, targeted treatment, which focuses only on persons with active infection and their contacts, may not achieve a sufficient level of treatment coverage among those with latent infection.[12] On the basis of both empirical and modeling data,[13,14] we hypothesized that three rounds of mass administration of azithromycin before switching to targeted treatment would be more effective in reducing the prevalence of active and latent yaws than standard care with one round of mass administration of azithromycin.

METHODS

TRIAL SETTING AND PARTICIPANTS

From April 2018 through October 2019, we conducted a phase 3, cluster-randomized, open-label, community-based trial in the Namatanai District of the New Ireland Province of Papua New Guinea. The district consists of six local-level government areas, three of which were selected for this trial: Matalai Rural, Namatanai Rural, and Sentral Niu Ailan Rural. The three local-level government areas are subdivided into 43 wards, of which we excluded five owing to remoteness. Wards are the smallest administrative unit, encompassing a group of three to five villages that share the same school, church, or both. For the purpose of this trial, each cluster comprised all eligible persons who were residents of the same ward. On the basis of a census conducted in 2016, with correction for population growth, the population of the 38 wards of the three local-level government areas was estimated to be 56,676 residents.[15] We randomly assigned 38 wards to the experimental group or the control group. All persons older than 1 month and living in the trial wards were eligible to participate. Exclusion criteria for receiving azithromycin were known allergy to macrolide antibiotics and severe illness.

Thirty-eight field teams (one in each ward) were responsible for delivering the treatment intervention and collecting trial data. Field teams were selected by the Papua New Guinea National Department of Health and consisted of a team leader (either a community health worker or a nurse) and four community health volunteers. Village leaders from the trial wards were engaged to serve as gatekeepers for entry into communities and ensure participant recruitment and retention. Villagers were informed in advance about the date of the trial visit and were invited to visit a central location in the village for assessment and treatment by means of "Toksave" (public notice in Tok Pisin, the most widely spoken language in Papua New Guinea).

All the trial participants — or, for children, their parent or guardian — provided oral informed consent for screening and treatment. Participants with skin lesions that were suspected to be attributable to yaws provided written informed consent for the collection of biologic samples. The trial protocol, available with

the full text of this article at NEJM.org, was approved by the Medical Research Advisory Committee of the Papua New Guinea National Department of Health, which authorized the oral consent by the participants.

The authors vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol. The funders (Fundació "la Caixa," Diputació de Barcelona, and Fundació Barberà Solidària) had no role in the design of the trial; in the collection, analysis, and interpretation of the data; in the writing of the manuscript; or in the decision to submit the manuscript for publication. The corresponding author had full access to all the data in the trial and had final responsibility for the decision to submit the manuscript for publication.

RANDOMIZATION AND INTERVENTIONS

The 38 wards were randomly assigned in a 1:1 ratio to the control group or the experimental group. Randomization was stratified according to the local-level government area with the use of a computer-generated random-assignment sequence. The control group received a single round of mass administration of azithromycin at baseline, followed by two rounds of targeted treatment at 6 months and 12 months according to the Morges strategy, and the experimental group received three rounds of mass administration of azithromycin (one round at baseline, one at 6 months, and one at 12 months). Mass drug administration consisted of blanket treatment irrespective of infection status. Targeted treatment consisted of proactive screening of all participants for evidence of yaws; participants with yaws-like lesions and their household and any school contacts were offered treatment.

Azithromycin (Kern Pharma) was administered orally as a single dose, with the participant under direct observation; the dose was adjusted according to age, as recommended in the WHO guidelines: persons 15 years of age or older received four 500-mg tablets in each round, those aged 10 to 14 years received three 500-mg tablets, those aged 5 to 9 years received two 500-mg tablets, and those aged 1 to 4 years received one 500-mg tablet or 7.5 ml of azithromycin syrup. Azithromycin syrup (5 ml) was offered to children aged 1 to 12 months. Azithromycin was donated by Kern Pharma and was provided at no cost to the trial participants.

The implementation of each treatment round, either mass drug administration or targeted treatment, lasted 5 days. On the first 3 days, villagers were invited to visit a central location for assessment and treatment. On the subsequent 2 days, the field teams conducted house-to-house visits to find residents who had not been treated at the central points. Census and tally sheets of the population surveyed were used to assess coverage.

END POINTS AND PROCEDURES

The coprimary end points were the prevalence of active cases of yaws, confirmed by polymerase-chain-reaction (PCR) assay, in the entire trial population and the prevalence of latent yaws, confirmed by serologic testing, in a subgroup of asymptomatic children aged 1 to 15 years; these prevalences were measured at the 18-month survey. The prevalence of active yaws was also measured at baseline and at the 6-month and 12-month surveys. Secondary end points included the genetic diversity of yaws, the percentage of macrolide-resistant yaws strains, and the prevalence of ulcers caused by *Haemophilus ducreyi*, which coexists with yaws as a cause of skin ulcers and responds well to azithromycin.[16]

Clinical surveys of the prevalence of active yaws were undertaken in the entire resident population. The clinical definition of active yaws was an ulcerative or nodular skin lesion larger than 1 cm in diameter. A swab specimen from the lesion was obtained for each patient and shipped to Masaryk University (Czech Republic) for detection of *T. pallidum* subspecies *pertenue* DNA, strain genotyping, identification of mutations associated with resistance to azithromycin, and detection of *H. ducreyi* DNA. Methods for the collection of clinical and demographic data and laboratory analyses are described in the Supplementary Appendix, available at NEJM.org. PCR was performed by means of a nested-PCR technique. As described previously,[17] allelic profiles based on sequences of the genes *TP0548*, *TP0488*, and *TP0858* (Table S1 in the Supplementary Appendix) were assigned to individual samples and used to determine the mean evolutionary diversity of *T. pallidum* subspecies *pertenue* at each time point; the presence of the point mutations A2058G and A2059G in the 23S ribosomal RNA (rRNA) genes causing macrolide resistance were identified by sequencing; and all samples were screened by means of quantitative PCR targeting

H. ducreyi.[18] Laboratory personnel who performed the assessment were unaware of the treatment-group assignments.

In each treatment group, 12 of 19 clusters (i.e., wards) were randomly selected to measure the prevalence of latent yaws at 18 months. In each cluster, we randomly selected a sample of 75 asymptomatic children aged 1 to 15 years, totaling 900 children per group. Latent yaws was assessed by means of finger-prick rapid quantitative serologic testing (Dual Path Platform Syphilis Screen and Confirm assay, Chembio Diagnostics).[19] A serologic test for yaws was considered to be positive if both the treponemal antibody assay and the nontreponemal antibody assay showed reactivity, with optical densities of at least 12 and at least 30, respectively.[20] A high-titer serologic response was defined as an optical density of at least 90 on the nontreponemal assay.

STATISTICAL ANALYSIS

On the basis of a previous public health intervention for yaws elimination,[7] we expected a 0.11% prevalence of PCR-confirmed active yaws in the control group at 18 months and a 0.75 coefficient of variation of the ward-level prevalence. The trial was originally powered for the coprimary end points in 43 wards in the trial area; the harmonic mean of the estimated population per ward was 1177 (according to a census conducted in the year 2000 — the only one we had access to in the early stages of trial design), and we expected that 80% of the population would be screened during clinical surveys. On the basis of these assumptions, the trial would have 77.3% power to detect an 80% lower prevalence of PCR-confirmed active yaws in the experimental group than in the control group at 18 months at a two-sided alpha significance level of 0.05. Ultimately, five island wards were excluded because of remoteness, and the revised power analysis that considered the reduction to 38 wards and that used a more recent census conducted in 2016 (harmonic mean of population per ward, 1302) showed that the trial would have 75.7% power.

With respect to latent yaws, we expected a 6% prevalence among children in the control group and a 0.30 coefficient of variation of the ward-level prevalence. On the basis of these assump-tions, 1800 persons would provide the trial 80% power to detect a 50% lower prevalence of latent yaws in the experimental group than in the control group at 18 months at a two-sided alpha significance level of 0.05.

The primary analysis included all the participants who resided in the trial wards, met the selection criteria, and were present at the 18-month clinical survey. Prevalence with two-sided confidence intervals was calculated by means of the Wald method.[21] For the coprimary end points, we had originally planned to estimate odds ratios, but we used relative risks instead, because they are less likely to overestimate risks and are easier to interpret. Finally, we fitted a generalized estimating equation log-binomial model that accounted for clustering[22] to estimate a relative risk comparing the prevalence of PCR-confirmed active yaws in the experimental group with that in the control group. To determine whether the log-relative risk of PCR-confirmed yaws between groups was significantly different from zero at a two-sided alpha level of 0.05, we tested the fitted treatment-effect coefficient with a Wald test using the robust standard error. We used the same methods for the latent yaws end point. We did not adjust the type I error for multiplicity because we considered that each coprimary end point must show a significant treatment benefit. Coverage rates were calculated with the use of tally sheets of the population surveyed in each treatment round (numerator) and estimates of the eligible population based on the 2016 census adjusted for population growth (denominator).[15] We applied a 5% yearly growth factor to obtain the 2018-corrected population. The statisticians were unaware of the treatment-group assignments and were allowed access only to coded group data. All statistical analyses were performed with the use of R software, version 3.6.3 (R Foundation for Statistical Computing).

RESULTS

TRIAL POPULATION AND COVERAGE

The trial population comprised 56,676 residents of the 38 wards that were randomly assigned to the control group (19 wards; 30,438 participants) or the experimental group (19 wards; 26,238 participants) (Fig. 1). The distribution of partici-
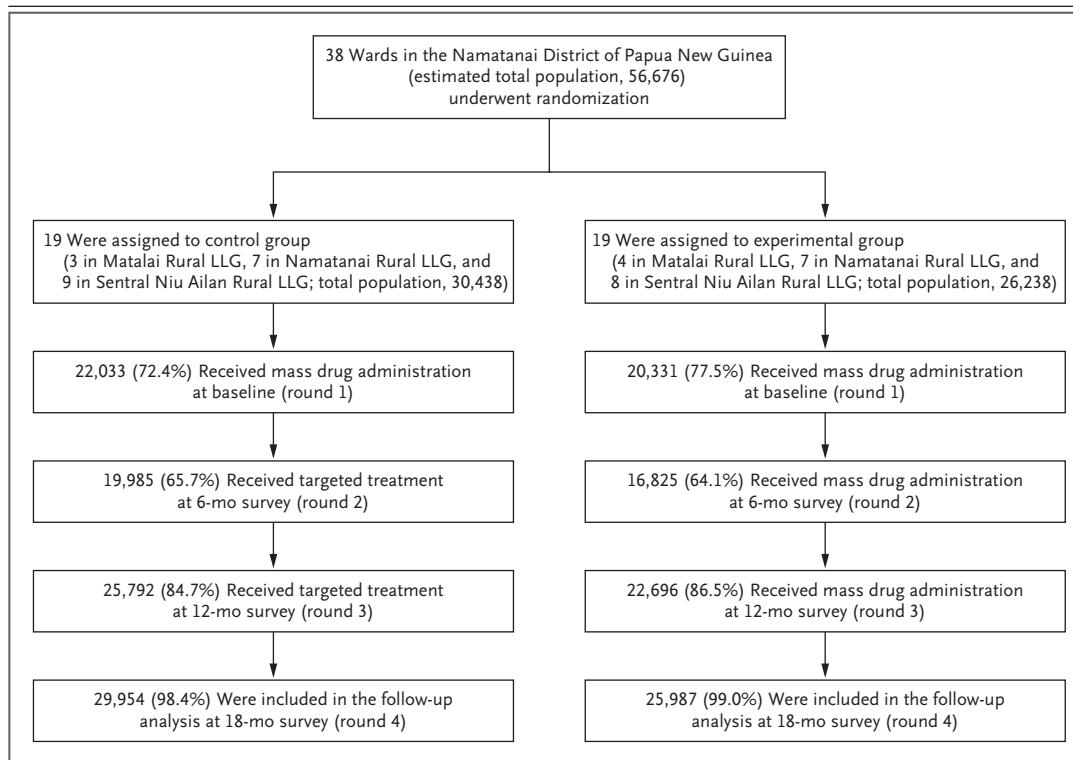
Figure 1. Randomization, Treatment Rounds, and Follow-up.

The estimation of the total population was based on a survey conducted in 2016 for bed-net distribution. The trial area consisted of three local-level government (LLG) areas in the Namatanai District. Each LLG in the trial area has between 7 and 22 wards. We used the ward as the randomization unit to reduce the risk of spillover between treatment groups. Ward residents often attend the same school or church, but this is less common among residents of different wards. We selected 7 of 7 wards in Matalai Rural LLG, 14 of 14 wards in Namatanai Rural LLG, and 17 of 22 wards in Sentral Niu Ailan LLG. On the basis of a census conducted in 2016, with correction for population growth, the arithmetic mean population of the wards was 1491 (harmonic mean, 1302; range, 679 to 1902).[15] Five island wards of Sentral Niu Ailan were excluded because of remoteness. The coverage at the 6-month survey (October 2018) was lower owing to a tropical cyclone that caused roadblocks and reduced access to many villages.

pants according to age and sex at baseline was similar in the treatment groups (Table 1). A total of 42,362 participants (74.7%) received the intervention at baseline, 36,810 (64.9%) at 6 months, and 48,488 (85.6%) at 12 months. Coverage did not differ between the two treatment groups at any time point (Fig. 1). A total of 24,848 doses of azithromycin were administered in the control group (22,033 were given to the participants at round 1, and 207 and 2608 were given to the participants with yaws-like lesions and their contacts, respectively, at targeted-treatment rounds 2 and 3 [combined]), and 59,852 doses were administered in the experimental group. We were able to locate and assess 55,941 persons (98.7%) for the primary end-point assessment at 18 months.

CHANGES IN PREVALENCE OF ACTIVE YAWS

During the trial period, we identified 1026 ulcers, of which 297 were confirmed to be yaws on PCR assay (Table 2). At baseline, the prevalence of active yaws was 0.46% (102 of 22,033 participants) in the control group and 0.43% (87 of 20,331) in the experimental group. At the 18-month survey, the prevalence of active yaws decreased to 0.16% (47 of 29,954) in the control group and to 0.04% (10 of 25,987) in the experimental group (relative risk adjusted for clustering, 4.08; 95% confidence interval [CI], 1.90 to 8.76). The prevalence of active yaws was highest among children younger than 15 years of age (Fig. S2), and the rebound in the control group was particularly marked among children 6 to 10 years of age.
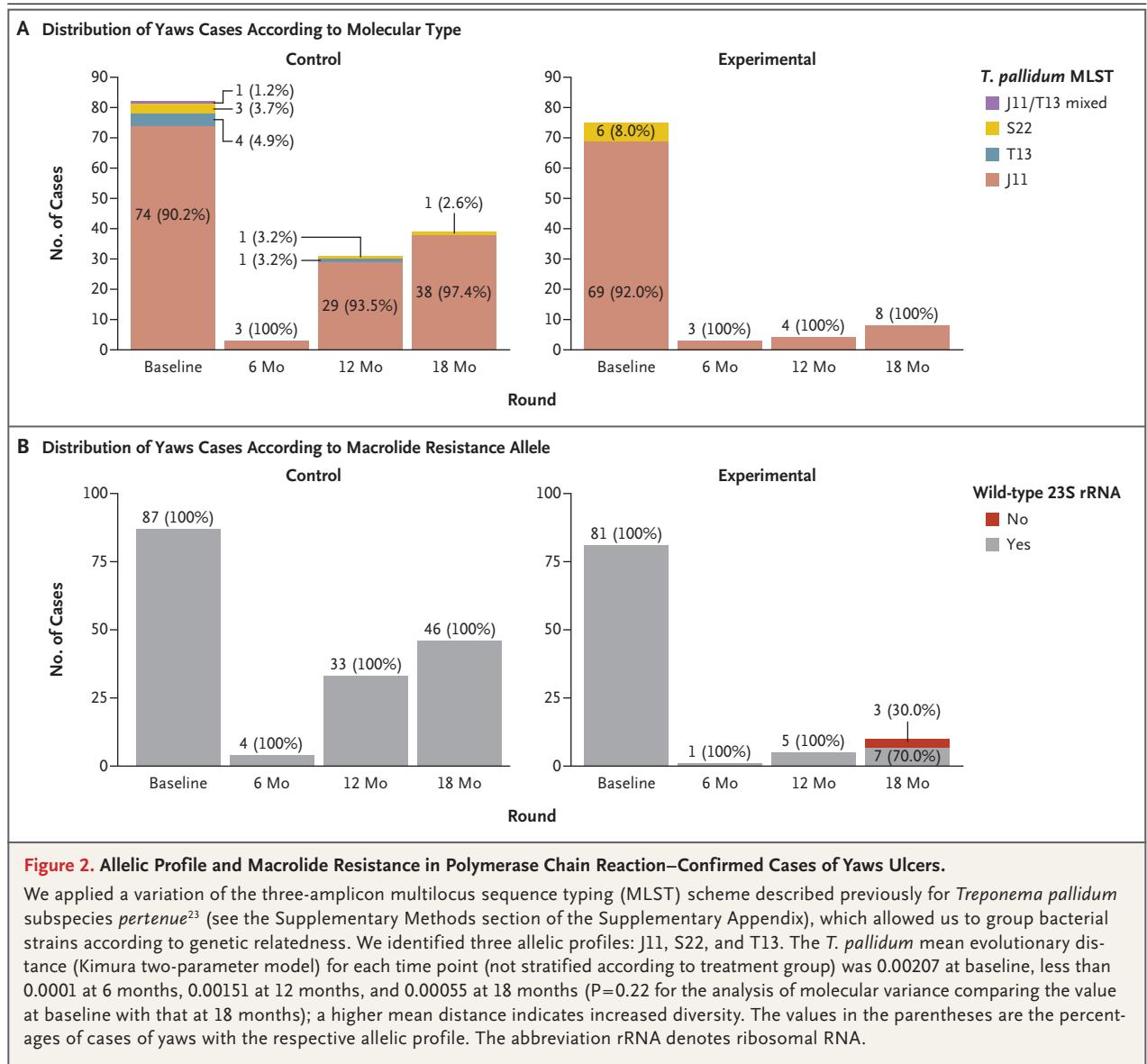
Table 1. Demographic Characteristics of the Participants at Baseline.

Characteristic	Total (N=42,364)	Control Group (N=22,033)	Experimental Group (N=20,331)
	number	*number (percent)*	
Local-level government			
Matalai Rural	6,810	2,813 (12.8)	3,997 (19.7)
Namatanai Rural	16,667	8,148 (37.0)	8,519 (41.9)
Sentral Niu Ailan Rural	18,887	11,072 (50.3)	7,815 (38.4)
Sex			
Female	20,311	10,556 (47.9)	9,755 (48.0)
Male	22,053	11,477 (52.1)	10,576 (52.0)
Age group			
≤5 yr	6,997	3,604 (16.4)	3,393 (16.7)
6–10 yr	5,348	2,763 (12.5)	2,585 (12.7)
11–15 yr	6,075	3,209 (14.6)	2,866 (14.1)
>15 yr	23,944	12,457 (56.5)	11487 (56.5)

Table 2. Prevalence of Ulcers with *Treponema pallidum* Subspecies *pertenue* DNA, with *Haemophilus ducreyi* DNA, and without DNA from Either Organism.*

Variable	Control Group		Experimental Group		Relative Risk Adjusted for Clustering (95% CI)†
	Participants	Prevalence (95% CI)	Participants	Prevalence (95% CI)	
	no.	*%*	*no.*	*%*	
T. pallidum subspecies *pertenue*					
Baseline	102	0.46 (0.38–0.55)	87	0.43 (0.34–0.52)	1.08 (0.61–1.92)
6 mo	5	0.03 (0.01–0.05)	3	0.02 (0.00–0.04)	1.40 (0.13–14.77)
12 mo	38	0.15 (0.10–0.20)	5	0.02 (0.00–0.04)	6.69 (2.05–21.79)
18 mo	47	0.16 (0.11–0.20)	10	0.04 (0.02–0.07)	4.08 (1.90–8.76)‡
H. ducreyi					
Baseline	52	0.24 (0.17–0.30)	67	0.33 (0.25–0.41)	0.72 (0.31–1.66)
6 mo	7	0.04 (0.01–0.07)	1	0.01 (0.00–0.02)	5.89 (0.69–50.01)
12 mo	54	0.21 (0.16–0.27)	17	0.07 (0.04–0.11)	2.80 (0.87–9.01)
18 mo	40	0.13 (0.09–0.18)	33	0.13 (0.08–0.17)	1.05 (0.53–2.10)
Non–*T. pallidum* subspecies *pertenue*, non–*H. ducreyi* ulcers					
Baseline	79	0.36 (0.28–0.44)	84	0.41 (0.33–0.50)	0.87 (0.53–1.41)
6 mo	43	0.22 (0.16–0.28)	44	0.26 (0.19–0.34)	0.82 (0.32–2.08)
12 mo	60	0.23 (0.17–0.29)	8	0.04 (0.01–0.06)	6.60 (1.95–22.32)
18 mo	67	0.22 (0.17–0.28)	73	0.28 (0.22–0.35)	0.80 (0.42–1.53)

* Detection was performed with the use of polymerase-chain-reaction assay. CI denotes confidence interval.
† A log-binomial model was applied to estimate the relative risk.
‡ P<0.001 for the coprimary end point of the prevalence of active yaws (i.e., detection of *T. pallidum* subspecies *pertenue* at 18 months).

Figure 2. Allelic Profile and Macrolide Resistance in Polymerase Chain Reaction–Confirmed Cases of Yaws Ulcers.

We applied a variation of the three-amplicon multilocus sequence typing (MLST) scheme described previously for *Treponema pallidum* subspecies *pertenue*[23] (see the Supplementary Methods section of the Supplementary Appendix), which allowed us to group bacterial strains according to genetic relatedness. We identified three allelic profiles: J11, S22, and T13. The *T. pallidum* mean evolutionary distance (Kimura two-parameter model) for each time point (not stratified according to treatment group) was 0.00207 at baseline, less than 0.0001 at 6 months, 0.00151 at 12 months, and 0.00055 at 18 months (P=0.22 for the analysis of molecular variance comparing the value at baseline with that at 18 months); a higher mean distance indicates increased diversity. The values in the parentheses are the percentages of cases of yaws with the respective allelic profile. The abbreviation rRNA denotes ribosomal RNA.

The molecular biologic findings of PCR-confirmed yaws ulcers sampled in each survey are shown in Figure 2 (additional clinic and serologic findings are shown in Table S3). With the use of multilocus sequence typing, three different allelic profiles were identified: J11, S22, and T13 (corresponding to the JG8, SE7, and TD6 profiles that were described previously).[23] On the basis of multilocus sequence typing of 157 cases at baseline, 91.1% (143 cases) had the J11 profile, 5.7% (9 cases) the S22 profile, 2.5% (4 cases) the T13 profile, and 0.6% (1 case) a mixed J11/T13 profile. Molecular diversity decreased from baseline to 6 months (J11 in 100% [6 of 6] of cases)

and then remained lower at 12 months and 18 months than at baseline (J11 in 94.3% [33 of 35] and 97.9% [46 of 47], respectively) (P=0.22 for the analysis of molecular variance comparing the value at baseline with that at 18 months). All the cases of yaws had a wild-type 23S rRNA, except for 3 cases at the 18-month survey in the experimental group, which had the A2058G mutation associated with macrolide resistance.

OTHER ULCERATIVE PATHOGENS

Of the 729 non-yaws ulcers, 271 (37.2%) had a positive PCR assay for *H. ducreyi* and 458 (62.8%) were non–*T. pallidum* subspecies *pertenue*, non–

Table 3. Prevalence of Latent Yaws at 18 Months.*

Outcome	Control Group (N=994)		Experimental Group (N=945)		Relative Risk Adjusted for Clustering (95% CI)†	Relative Risk Adjusted for Clustering and Age (95% CI)†
	Participants	Prevalence (95% CI)	Participants	Prevalence (95% CI)		
	no.	%	no.	%		
Reactive treponemal assay	433	43.56 (40.48–46.64)	322	34.07 (31.05–37.10)	1.29 (0.86–1.93)	1.31 (0.88–1.97)
Positive serologic test	65	6.54 (5.00–8.08)	31	3.28 (2.14–4.42)	1.99 (1.10–3.61)	2.03 (1.12–3.70)‡
High-titer serologic response	14	1.41 (0.68–2.14)	5	0.53 (0.07–0.99)	2.66 (0.98–7.23)	2.58 (0.92–7.22)

* The prevalence of latent yaws at 18 months was assessed in a subgroup of asymptomatic children aged 1 to 15 years with the use of a Dual Path Platform Syphilis Screen and Confirm assay (Chembio Diagnostics) on a capillary blood sample from the finger. Optical densities (ODs) of at least 30 and at least 90 on the nontreponemal assay correspond to rapid plasma reagin titers of 1:4 and 1:16, respectively.[20] A participant was considered to have a reactive treponemal assay if the OD on the treponemal assay was at least 12, a positive serologic test for yaws if the OD on the treponemal assay was at least 12 and the OD on the nontreponemal assay was at least 30, and a high-titer serologic response if the OD on the treponemal assay was at least 12 and the OD on the nontreponemal assay was at least 90.
† A log-binomial model was applied to estimate the relative risk.
‡ P=0.02 for the coprimary end point of a positive serologic test (i.e., the prevalence of latent yaws at 18 months).

H. ducreyi ulcers. At baseline, the prevalence of ulcers caused by *H. ducreyi* was 0.24% (95% CI, 0.17 to 0.30) in the control group and 0.33% (95% CI, 0.25 to 0.41) in the experimental group. The prevalence decreased at 6 months and returned to values similar to those at baseline in both treatment groups at 18 months (Table 2). The first round of mass administration of azithromycin had a mild effect on non–*T. pallidum* subspecies *pertenue*, non–*H. ducreyi* ulcers, and the prevalence at 18 months was similar to that at baseline in both treatment groups.

CHANGES IN THE PREVALENCE OF LATENT YAWS

The serosurvey for latent yaws at 18 months was conducted among 994 children in the control group and 945 children in the experimental group (Table 3). The prevalence of positive serologic tests (i.e., optical densities of ≥12 on the treponemal assay and ≥30 on the nontreponemal assay) was 6.54% (95% CI, 5.00 to 8.08) in the control group and 3.28% (95% CI, 2.14 to 4.42) in the experimental group (relative risk adjusted for clustering and age, 2.03; 95% CI, 1.12 to 3.70). The prevalence of a high-titer serologic response (i.e., optical density of ≥90 on the nontreponemal assay) was 1.41% (95% CI, 0.68 to 2.14) in the control group and 0.53% (95% CI, 0.07 to 0.99) in the experimental group (relative risk adjusted for clustering and age, 2.58; 95% CI, 0.92 to 7.22).

DISCUSSION

In this cluster-randomized trial, we found a reduction in the prevalence of active yaws after one round of mass administration of azithromycin that was in line with previous mass treatment interventions.[7-9] In clusters that received two additional rounds of mass administration of azithromycin, the prevalence remained low at 18 months as compared with those that received only a single round of mass drug administration followed by targeted treatment rounds. This finding is supported by a lower seroprevalence of infection among asymptomatic children in the experimental group than among those in the control group, an outcome consistent with a reduction in the burden of latent yaws. As anticipated, substantially more participants were treated with azithromycin in the experimental group. Over the 18-month period, 500 extra doses of

azithromycin were administered for each case of active yaws prevented and approximately 100 extra doses were administered for each case of latent yaws avoided among children.

Three consecutive rounds of mass drug administration reduced the prevalence of both active and latent yaws but did not get all the way to elimination. This result could be due to a spillover of cases from the wards in the control group to the wards in the experimental group, an insufficient number of latent cases treated, or active cases that were missed. An assessment of this outcome is difficult because we did not have longitudinal information at the individual level for the whole population. In addition, we found the A2058G mutation, suggestive of macrolide-resistant strains of *T. pallidum* subspecies *pertenue*, in three participants in the experimental group. The frequency of resistance was similar to what we observed after repeated targeted-treatment rounds in the nearby island of Lihir.[10,24,25]

Similar to other PCR-based studies of ulcerative skin lesions in areas with a high incidence of yaws,[26,27] this study showed a high percentage of ulcers caused by microorganisms other than *T. pallidum* subspecies *pertenue*. Patients with ulcers caused by *H. ducreyi* also benefited from the initial round of mass administration of azithromycin, but the prevalence of these ulcers rebounded in subsequent rounds. The overall community prevalence of ulcers of unknown cause (a large fraction of which has been previously suggested to be caused by *Streptococcus pyogenes*[28]) was unchanged after mass administration of azithromycin. This finding underscores the importance of nonpharmacologic strategies (e.g., hygiene and sanitation) for the epidemiologic control of skin ulcers.[29]

Our trial has several strengths as compared with previous studies. First, the cluster-randomized trial design provided evidence that the decrease in the prevalence of both active and latent yaws was attributable to the experimental intervention. Second, the generalizability of our findings from a subdistrict located in a large landmass with contiguous nontreated subdistricts is greater than that of previous efforts to reduce the community prevalence of yaws on smaller islands. Third, the intervention was performed within the structures of the routine health system with local resources at the community level and was lead by the National Department of Health. However, our trial has several limitations, including the lack of an updated population census. Furthermore, we cannot comment on whether a different number of rounds of mass drug administration might have been more effective. It would have been helpful in this regard to assess whether counts stayed low in the experimental group beyond 6 months after the last round of mass drug administration, but this could not be assessed because of travel restrictions related to coronavirus disease 2019. In addition, we did not assess for the emergence of macrolide resistance in other organisms.

Our data suggest that more than one round of mass drug administration should be considered as part of the strategy for yaws eradication. The selection and spread of antimicrobial resistance and the failure to eliminate yaws in the area of our trial after three consecutive rounds of mass drug administration highlight the need to maintain careful clinical and molecular surveillance for the emergence of antimicrobial resistance in *T. pallidum* subspecies *pertenue* and other bacterial organisms associated with mass drug administration.[13]

APPENDIX

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REFERENCES

1. Mitjà O, Asiedu K, Mabey D. Yaws. Lancet 2013;381:763-73.
2. Fitzpatrick C, Asiedu K, Jannin J. Where the road ends, yaws begins? The cost-effectiveness of eradication versus more roads. PLoS Negl Trop Dis 2014; 8(9):e3165.
3. Mitjà O, Hays R, Ipai A, et al. Single-dose azithromycin versus benzathine benzylpenicillin for treatment of yaws in children in Papua New Guinea: an open-label, non-inferiority, randomised trial. Lancet 2012;379:342-7.
4. World Health Organization. Summary report of a consultation on the eradication of yaws: 5–7 March 2012, Morges, Switzerland. 2012 (https://apps.who.int/iris/handle/10665/75528).
5. World Health Organization. Eradication of yaws: a guide for programme managers. 2018 (https://apps.who.int/iris/bitstream/handle/10665/259902/9789241512695-eng.pdf).
6. World Health Organization. Yaws. May 17, 2021 (https://www.who.int/news-room/fact-sheets/detail/yaws).
7. Mitjà O, Houinei W, Moses P, et al. Mass treatment with single-dose azithromycin for yaws. N Engl J Med 2015;372: 703-10.
8. Marks M, Sokana O, Nachamkin E, et al. Prevalence of active and latent yaws in the Solomon Islands 18 months after azithromycin mass drug administration for trachoma. PLoS Negl Trop Dis 2016; 10(8):e0004927.
9. Abdulai AA, Agana-Nsiire P, Biney F, et al. Community-based mass treatment with azithromycin for the elimination of yaws in Ghana — results of a pilot study. PLoS Negl Trop Dis 2018;12(3):e0006303.
10. Mitjà O, Godornes C, Houinei W, et al. Re-emergence of yaws after single mass azithromycin treatment followed by targeted treatment: a longitudinal study. Lancet 2018;391:1599-607.
11. Marks M, Mitjà O, Fitzpatrick C, et al. Mathematical modeling of programmatic requirements for yaws eradication. Emerg Infect Dis 2017;23:22-8.
12. Dyson L, Marks M, Crook OM, et al. Targeted treatment of yaws with household contact tracing: how much do we miss? Am J Epidemiol 2018;187:837-44.
13. World Health Organization. Report of a global meeting on yaws eradication surveillance, monitoring and evaluation: Geneva, 29–30 January 2018. December 19, 2018 (https://www.who.int/publications/i/item/WHO-CDS-NTD-IDM-2018.08?).
14. Holmes A, Tildesley MJ, Solomon AW, et al. Modeling treatment strategies to inform yaws eradication. Emerg Infect Dis 2020;26:2685-93.
15. Hetzel MW, Saweri OPM, Kuadima JJ, et al. Papua New Guinea malaria indicator survey 2016–2017: malaria prevention, infection, and treatment. Goroka: Papua New Guinea Institute of Medical Research, 2018 (https://www.malariasurveys.org/documents/PNGIMR%202018%20-%20PNG%20MIS%202016-17%20-%20Final%20Report%2006.04.2018.pdf).
16. González-Beiras C, Kapa A, Vall-Mayans M, et al. Single-dose azithromycin for the treatment of *Haemophilus ducreyi* skin ulcers in Papua New Guinea. Clin Infect Dis 2017;65:2085-90.
17. Grillová L, Bawa T, Mikalová L, et al. Molecular characterization of *Treponema pallidum* subsp. *pallidum* in Switzerland and France with a new multilocus sequence typing scheme. PLoS One 2018; 13(7):e0200773.
18. Orle KA, Gates CA, Martin DH, Body BA, Weiss JB. Simultaneous PCR detection of Haemophilus ducreyi, Treponema pallidum, and herpes simplex virus types 1 and 2 from genital ulcers. J Clin Microbiol 1996;34:49-54.
19. Marks M, Yin Y-P, Chen X-S, et al. Metaanalysis of the performance of a combined treponemal and nontreponemal rapid diagnostic test for syphilis and yaws. Clin Infect Dis 2016;63:627-33.
20. Ayove T, Houniei W, Wangnapi R, et al. Sensitivity and specificity of a rapid point-of-care test for active yaws: a comparative study. Lancet Glob Health 2014;2(7):e415-e421.
21. Julious SA. Two-sided confidence intervals for the single proportion: comparison of seven methods by Robert G. Newcombe, Statistics in Medicine 1998; 17:857-872. Stat Med 2005;24:3383-4.
22. Cui J. QIC program and model selection in GEE analyses. Stata J 2007;7:209-20.
23. Godornes C, Giacani L, Barry AE, Mitja O, Lukehart SA. Development of a multi-locus sequence typing (MLST) scheme for *Treponema pallidum* subsp. *pertenue*: application to yaws in Lihir Island, Papua New Guinea. PLoS Negl Trop Dis 2017;11(12): e0006113.
24. Šmajs D, Pospíšilová P. Macrolide resistance in yaws. Lancet 2018;391:1555-6.
25. Beale MA, Noguera-Julian M, Godornes C, et al. Yaws re-emergence and bacterial drug resistance selection after mass administration of azithromycin: a genomic epidemiology investigation. Lancet Microbe 2020;1(6):e263-e271.
26. Mitjà O, Lukehart SA, Pokowas G, et al. Haemophilus ducreyi as a cause of skin ulcers in children from a yaws-endemic area of Papua New Guinea: a prospective cohort study. Lancet Glob Health 2014; 2(4):e235-e241.
27. Ndzomo Ngono J-P, Tchatchouang S, Noah Tsanga MV, et al. Ulcerative skin lesions among children in Cameroon: it is not always yaws. PLoS Negl Trop Dis 2021; 15(2):e0009180.
28. Griesenauer B, González-Beiras C, Fortney KR, et al. Streptococcus pyogenes is associated with idiopathic cutaneous ulcers in children on a yaws-endemic island. mBio 2021;12(1):e03162-220.
29. G-Beiras C, Ubals M, Corbacho-Monné M, Vall-Mayans M, Mitjà O. Yaws, *Haemophilus ducreyi*, and other bacterial causes of cutaneous ulcer disease in the South Pacific islands. Dermatol Clin 2021;39:15-22.