Re-emergence of yaws after single mass azithromycin treatment followed by targeted treatment: a longitudinal study

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ABSTRACT

Background

Yaws is a substantial cause of chronic disfiguring ulcers in children in at least 14 countries in the tropics. WHO's newly adopted strategy for yaws eradication employs a single round of mass azithromycin treatment followed by targeted treatment programs, and data from pilot studies have shown a short-term significant reduction of yaws. We assessed the long-term efficacy of the WHO strategy for yaws eradication.

Methods

Between April 15, 2013, and Oct 24, 2016, we did a longitudinal study on a Papua New Guinea island (Lihir; 16092 population) in which yaws was endemic. In the initial study, the participants were followed for 12 months; in this extended follow-up study, clinical, serological, and PCR surveys were continued every 6 months for 42 months. We used genotyping and travel history to identify importation events. Active yaws confirmed by PCR specific for *Treponema pallidum* was the primary-outcome indicator. This study is registered with ClinicalTrials.gov, number NCT01955252.

Findings

Mass azithromycin treatment (coverage rate of 84%) followed by targeted treatment programs reduced the prevalence of active yaws from 1·8% to a minimum of 0·1% at 18 months (difference from baseline, -1·7%, 95% CI, -1.9 to -1.4; P<0·0001), but the infection began to re-emerge after 24 months with a significant increase to 0·4% at 42 months (difference from 18 months, 0·3%, 95% CI 0.1 to 0.4; P<0·0001). At each time point after baseline, > 70% of the total community burden of yaws was found in persons who had not had the mass treatment or as new infections in non-travelling residents. At months 36 and 42, five cases of active yaws, all from the same village, demonstrated clinical failure following azithromycin treatment, with PCR detected mutations in the 23S ribosomal RNA genes conferring resistance to azithromycin. A sustained decrease in the prevalence of high titre latent yaws from 13.7% to <1.5% in asymptomatic children aged 1–5 years old and of genetic diversity of yaws strains from 0·139 to <0·046 between 24 and 42 months indicated a reduction in transmission of infection.

Interpretation

The implementation of the WHO strategy did not, in the long-term, achieve elimination in a high-endemic community mainly due to the individuals who were absent at the time of mass treatment in whom yaws reactivated; repeated mass treatment might be necessary to eliminate yaws. To our knowledge this is the first report of the emergence of azithromycin-resistant *T. p. pertenue* and spread within one village. Communities' surveillance should be strengthened to detect any possible treatment failure and biological markers of resistance.

Funding

ISDIN laboratories, Newcrest Mining Limited, andUS Public Health Service National Institutes of Health.

Panel 1. Research in context

Evidence before this study

We searched PubMed on Feb 17, 2017 for studies published in English using the search terms "yaws", "*Treponema pallidum*", "mass treatment", "azithromycin", and "penicillin". We searched for studies that assessed the efficacy of mass azithromycin treatment for yaws. Empirical data on the short-term impact of the WHO strategy became available after the start of this study; a single round of mass treatment in communities with high baseline infection rates in Ghana and Solomon Islands resulted in a significant decrease in prevalence of active and latent yaws at 12 and 18 months after treatment, respectively. The quality of the evidence was low, comprising primarily small sample size cross-sectional studies. Moreover, previous experience in the 1950s using mass treatment with penicillin and recent mathematical modelling suggested that, after an initial reduction, the disease may persist or rebound to pre-mass treatment amounts.

Added value of this study

To our knowledge, this is the first study to report the long-term efficacy of the modern WHO strategy for yaws eradication. We repeatedly examined a community of about 16,000 people and used PCR analysis specific to *Treponema pallidum* to determine the prevalence of active disease over 42 months. Through this approach we were able to accurately measure the prevalence of active yaws and detect the appearance of macrolide-resistant strains. We used a novel genotyping method to determine temporal changes on genetic diversity of *T. p. pertenue* strains and to identify importation events.

Implications of all the available evidence

Active and latent yaws can be cured with single-dose azithromycin treatment and a single round of mass antibiotic treatment with coverage as high as 84% greatly reduces infection and transmission, but does not achieve complete and permanent reduction to zero new cases, with evidence of re-emergence after 24 months. We suggest that yaws eradication policies should be revised with consideration of expansion to repeated 6-12 monthly mass treatments for at least two-three rounds. The data also suggest that azithromycin resistance in *T. p. pertenue* has emerged as a result of the program implementation, which reveals the need for effective drug resistance monitoring as part of yaws eradication programs to prevent spread of antibiotic-resistant strains.

INTRODUCTION

The World Health Organization (WHO) has already begun implementing a program designed to eradicate yaws, an infectious disease caused by *Treponema pallidum* subsp. *pertenue* (*T p pertenue*). The global burden of yaws is substantial, with more than 89 million people living in yaws endemic areas and 100,000 annual reported cases of chronic ulcers or papilloma that are a major physical and psychological burden in young children.

The cornerstone of the WHO's strategy is the mass administration of azithromycin aiming for a population coverage of >90%.[1] The drug is well-tolerated and very effective against yaws.[2,3] It is given as one supervised dose, so compliance is assured. Treatment of all members of a yaws-endemic community, irrespective of their clinical status, allows individuals harbouring the infection without any skin manifestation (latent infection) to be successfully exposed to curative doses of the treatment. Clearance of the pathogen responsible for yaws from individuals with active and latent infection, which constitute the infectious reservoir, has the potential to interrupt transmission.[4] The WHO strategy calls for the use of PCR technology to confirm the diagnosis and to monitor the emergence of resistance to azithromycin after mass treatment.[5]

A second important element of the WHO strategy to increase the effectiveness of yaws eradication programmes is to follow mass drug administration (MDA) with active case detection surveys every 3-6 months, consisting of blanket screening to identify and treat all active yaws cases and their contacts (often called total targeted treatment).[6] This second element aims to achieve elimination by early detection of existing (e.g. missed MDA), recurrent (e.g. relapse of untreated latent infections), or newly introduced (e.g. crossing regional borders) active yaws cases. A third element is a strengthened health and community system for surveillance and management of patients who present to health care between surveys.[6]

We previously reported the effect of single-dose mass azithromycin treatment, with a coverage rate of 83·8%, on the prevalence of active and latent yaws 12 months after the intervention.[7] MDA with azithromycin was associated with a nearly 90% reduction of serologically-confirmed active yaws from 2·4% to 0·3%. Single mass azithromycin treatment has also shown short-term efficacy in other clinical trials in Ghana (Aziz A, West Akim District Health Administration, Eastern Region, Ghana, personal communication) and Solomon Islands;[8,9] however, the long-term efficacy of the WHO strategy has not yet been determined.

We now report the results of 42 months of follow-up in our study communities to assess the long-term effect of the WHO strategy to eradicate yaws.

METHODS

Study setting and participants

Between April 15, 2013 and Oct 24, 2016, we did a longitudinal study of yaws in the population of Lihir Island, New Ireland Province, Papua New Guinea. The characteristics of the area have been described in detail before.[7] The climate is tropical with two distinct seasons: rainy and dry seasons. All villages of Lihir Island had a high prevalence of active yaws before MDA (range 0·5 to 3·8%).[10]

We have previously reported the results of the first 12 months of follow up after MDA, in which the primary objective was to estimate the prevalence of clinically suspected yaws with serological confirmation of treponemal infection.[7] All residents of Lihir Island have been followed during the extended phase of this study for an additional 30 months (months 12–42). Serological methods do not result in identification of all cases of active yaws because very early infection, while highly infectious, can be seronegative. By contrast, participants with latent yaws can present with skin ulcers caused by other bacteria (e.g. *Haemophilus ducreyi*) resulting in false-positive diagnoses of

active yaws.[11-13] Consequently, throughout our study we incorporated molecular diagnostics as our case definition of active yaws. PCR testing of ulcers allowed us to more clearly delineate the effect of the intervention on participants with true active yaws (lesion PCR positive for *T. pallidum*) and on those participants with latent yaws and a different cause of the current skin infection (i.e. positive serology for treponemal infection but lesion PCR negative for *T. pallidum*).

The initial MDA programme consisted of a centralized distribution where community members in each village gathered in a central location and received treatment, and compliance was recorded in treatment register books (ie, census list). After the initial MDA campaign,[7] we did six monthly total targeted surveys in accordance with the standards advocated by WHO. Before each survey, population sensitization was undertaken to inform village authorities of the program. Villages were visited by a mobile team of health-care workers that first screened the village schools to examine children, and then did house-to-house screening. All subjects with skin ulcers and their contacts (household, frequent family friends, schoolmates, and playmates) were treated with directly observed single-dose azithromycin (30 mg/Kg) procured by WHO from Medopharm (Chennai, India). To simplify the treatment in the field, dosing charts were used to guide the participants' age-based dose. Individuals were observed for 30 minutes following treatment; if vomiting occurred within this time period, the child was retreated. Treatment was provided without cost to participants. Clinical follow-up examinations were conducted 2 weeks after any treatment to identify potential treatment failures. All field workers and clinical and laboratory staff involved in the follow-up of study participants remained masked to previous individual-specific and village-specific results.

All participants, or their parents, provided oral informed consent for screening. In addition, we obtained written informed consent from parents or guardians, as well as verbal agreement of the children with clinically suspected yaws before enrolment in ulcer aetiology studies and serological surveys. The protocol was approved by the National Medical Research Advisory Committee of the PNG Ministry of Health (MRAC n^{o} 12·36). The study is registered with ClinicalTrials.gov, number NTC01955252.

Procedures

Clinical surveys were undertaken in the entire resident population present at the time of the visits for assessment of clinical signs and symptoms of active yaws at study months 18, 24, 30, 36, and 42. We used tally sheets to record the number of people examined at total targerted treatment surveys and a standardized form to record data of patients with suspected lesions. Specimens were collected from the largest lesion of all subjects with ulcers or papillomas using dacron swabs (FITZCO Inc, Minneapolis, MN, USA) that were vigorously rotated across a 1 cm^2 area and then placed into a tube containing transport medium as previously described.[7] At the baseline and 6-month surveys we swabbe a systematic random sample of 90 and 84 ulcers. The specimens were forwarded to the University of Washington laboratory (Seattle, WA, USA) for PCR testing. The number of active yaws cases in the entire population was estimated using the proportion of PCR-positive specimens among the subset of PCR-tested lesions multiplied by the total number of detected lesions. In 12-month to 42-month surveys, we swabbed to test all ulcers detected; therefore we obtained a direct measurement of the number of yaws cases in the entire population.

Laboratory methods used to confirm yaws and to detect macrolide-resistance mutations have been previously described.[11,14] In short, three *T. pallidum* gene targets, *tp0548*, tpN47 (*tp0574*), and a *pertenue*-specific region of the tprL (*tp1031*) gene were PCR amplified to detect the presence of *T. pallidum* DNA and to confirm the subspecies. We used previously described restriction fragment length polymorphism methods to detect A2058G[14] and A2059G[15] point mutations in both copies of the 23S ribosomal RNA genes. We did strain genotyping by sequencing a panel of three molecular markers (*tp0548*, *tp0136* and *tp0326*)[16] to determine the genetic diversity of *T. p. pertenue*

infections and to identify importation events. The aggregated molecular typing of strains causing incident infection in Lihir Island has been reported in a paper[16] detailing the development of the typing system.

Significant reductions in transmission intensity are required to reduce the diversity of bacterial populations; hence we used genetic diversity as a marker for transmission. We used PCR targeting the 16S rRNA gene to identify *H. ducreyi.*[17] Participants with lesions underwent serological testing using both qualitative Rapid Plasma Reagin (RPR) and *T. pallidum* haemagglutination assay (TPHA); specimens which tested dually positive were analysed with the quantitative RPR.

Demographic and epidemiologic data were systematically collected for every case of ulcer detected. Compliance with yaws MDA therapy was assessed by self-reported data and verified using the treatment register books for MDA (ie, census lists). Travel history was assessed by self-reported travel out of Lihir Island to a yaws endemic area in the preceding 6 months, regardless of compliance to MDA. History of in-migration was assessed by self-reported migration to Lihir Island in the preceding 6 months and verified by non-appearance at the previous year census, regardless of compliance to MDA.

Serological surveys to detect latent yaws in a subgroup of asymptomatic children 1–15 years old were done at months 18, 24, 30, 36, and 42 after MDA. We selected six villages using computer-generated random numbers, and all children within the age group were recruited for inclusion. We regenerated the random sample at each survey; therefore different villages may have been selected for testing in different rounds. Venous blood samples were collected from assenting children for TPHA and qualitative and quantitative RPR testing.

Outcomes and statistical analysis

The primary outcome indicator to assess the prevalence of infection was the frequency of participants with active yaws lesions confirmed by PCR (regardless of their serology result), which was assessed by examining everyone in the population. Secondary outcome indicators included prevalence of participants with an ulcer who were serologically positive (regardless of PCR result). To control for potential confounders of infection persistence we looked at the proportion of post-MDA new incident yaws cases that had missed MDA therapy, the proportion of yaws cases that originated from travel versus local residual source according to travel history and genotyping results, and the proportion of yaws samples with genetic mutations associated with macrolide resistance at each timepoint. Secondary outcome indicators that were used to assess onward transmission of infection were the prevalence of latent yaws with high-titre seroreactivity (RPR ≥1:16) in a subset of children aged 1-5 years old in villages selected using computer generated random numbers, and the genetic diversity of *T. p. pertenue* isolates from active yaws lesions.

We estimated the prevalence ratiofor comparison of active yaws at seven time-points using a log-binomial regression model. The model accounted for the uncertainty in the estimate of number of active yaws cases at baseline. We estimated the adjusted prevalence ratio of high-titre latent yaws using the cluster option in the models to account for the variability between clusters selected for serosurveys. Analyses were done using Stata (version 13·1). To measure the genetic diversity of *T. p. pertenue* isolates at each round we estimated the Mean Evolutionary Diversity by calculating the number of base substitutions per site for each round using the Kimura 2 parameter model in MEGA (version 7).[18] We determined significant differences among years using one-way ANOVA.

We initially calculated that a sample size of 1000 children would be needed at 24-42 months to estimate the prevalence of high-titre latent yaws with a precision of 0·83%, at a two-sided significance level of 5% in a finite population of 6600 children 1-15 years old. We assumed that the

prevalence of latent yaws at 24-42 months would be 2%. However, we adjusted the sample size to reduce survey fatigue by the survey team and to minimize venipuncture of children; the revised calculations indicated that 500 children was enough to estimate prevalence with a precision of 1·18%.

RESULTS

The study population lives in the 28 villages of Lihir Island; in small subsistence farming communities with a mean population of 575 (SD 225) people per village. At baseline 16,092 people lived in the area, and a total of 13,490 individuals (83·8%) received single-dose azithromycin (or benzathine benzylpenicillin if azithromycin was contraindicated). Total population size remained fairly stable throughout the study, and a mean proportion of 79·0% (SD 8·2) of the population was examined at each survey (Table 1). Some individuals could not be reached at scheduled visit times (e.g. children were absent from school, adults were working in the fields, families had moved away on temporary or permanent basis).

The overall prevalence of active yaws fell from an estimated 1·8% before MDA to a minimum of 0·1% at 18 months (difference from baseline, -1·7%; 95%CI, -1·9 to -1·4; P<0·0001), but began to re-emerge from 30 months onwards (table 1). The prevalence increased to 0·4% at 42 months (difference from 18 months, 0·3%; 95%CI, 0·1 to 0·4; P<0·0001), with a major rise from 36 to 42 months (table 1). Similarly, the prevalence of participants with clinically suspected yaws lesions and positive serological findings fell initially, but the prevalence appeared to increaseby month 30 (table 1). We noted anincrease from a minimum of 0·2% at 24 months to 0·5% at 42 months (difference, 0·3%; 95CI, 0·1 to 0·4; P<0·0001).

Overall, 239 participants were PCR positive for *T. pallidum* throughout the study, including 31 (13%) at baseline and 208 (87%) in total in the period after MDA. At each survey after MDA, between 36% and 61% of the community burden of newly identified active yaws were accounted for by non-travelling residents who had been absent at initial mass treatment visit (figure-1). Non-travelling residents who were present at MDA ranged from 27% to 53%, and migrants and residents who had travelled to a yaws endemic area after MDA represented 28% of cases at each timepoint (figure 1).

We report the temporal variation of the genetic diversity over the 42-month study (figure 1). At baseline, three molecular types were identified, with strain JG8 accounting for ten (58·8%) of 17 fully typable samples (figure 1). Over time, molecular type diversity was reduced to zero (only one genotype –JG8– was present) at 24 months which represented a reduction of the mean evolutionary diversity of *T. p. pertenue* from 0·139 to 0·000 (P<0·0001; figure 1). Diversity remained low (<0.046) thereafter (figure 1). After 24 months, strain JG8 caused all 76 new incident cases of yaws in non-travelling patients with typable samples, and 18 (72%) of 25 cases in travelling patients. The remaining seven cases (28%) who had a history of travel or migration were infected with genotypes SE7 or SD6, which supports that these cases were imported and not derived from a local source.

When we looked at the proportion of participants with a history of travel according to genotypes (table 2), all TD6, TG6, and JD8, as well as 84% of JG8 specimens were seen in patients who had not travelled; all SD6 and TG8 strains, and 82% of SE7 occurred in patients who travelled or migrated.

Of 31 active yaws cases tested at baseline, the 23S rRNA gene could be amplified by PCR in 24 cases (77%); all had wild type 23S rRNA sequence at positions 2058 and 2059 (figure 1). Of 208 PCR-confirmed cases of active yaws in the period after MDA, the 23S rRNA gene could be amplified by PCR in 186 (89%), of which 181 (97%) had wild-type strains, but two cases (1%) at 36 months and three cases (2%) at 42 months revealed A2059G mutations associated with macrolide-resistance. The five samples had A2059G mutations in both of the 23S rRNA loci (figure 1).

The index case was an 11-year old boy who was diagnosed with active yaws at the 30-month survey (lesional swab was PCR-positive for *T. p. pertenue* with wild-type 23S rRNA),treated with a full dose of azithromycin 1.5 g without vomiting the medication, and shower clinical improvement 2 weeks after medication (figure 2). The patient was seen 6 months later with recurrent papillomatous lesions (figure 2) and serological treatment failure. The skin biopsy of these lesions showed abundant spirochetes (figure 2) and *T. p. pertenue* containing the 23S rRNA A2059G macrolide resistance mutation was identified by PCR. The patient was treated with 2.4 MUI of benzathine benzylpenicillin and showed clinical cure after 2 weeks and serological cure after 6 months.

The other four cases of antibiotic-resistant infection, diagnosed at 36 months (one case) and 42 months (three cases), were 9-14 year old boys who lived in the same village as the index case (related to or friends of the index case); they had not travelled outside the village. All of them reported no oral antibiotic-treatment other than that received during MDA. All presented with worsening skin lesions 2-weeks after azithromycin treatment and were subsequently treated with benzathine benzylpenicillin, according to age (1.2 MU for <10 years old and 2.4 MU for ≥10 years old). All strains with A2059G mutations were molecular type JG8, the most common type on Lihir Island.

The prevalence of high titre latent yaws in children aged 1–5 years fell from 14% before MDA to 1% at 12 months (P=0·0005)[7] and remained lower than 2% at each timepoint thereafter. No participant aged 1-5 years of age had high titre latent yaws infection at 30 or 42 months (Table 3). Decreases in this index were also observed in the older age group of 6–15 years. The decrease from baseline to 12 months was significant (P<0·0001). [7] At 18, 24, 36, and 42 months, the prevalence of high titre latent yaws infection remained significantly lower that it had been before mass treatment, although in each instance it was not significantly below that recorded for the previous round (table 3).

Regarding the proportion of ulcers due to *T. p. pertenue* and *H. ducreyi*, the baseline and 12 months after treatment results for PCR in detecting the causal agents have been previously described.[7] The proportion of subjects that had detectable *T. p. pertenue* DNA (either alone or co-infection with *H. ducreyi*) was lower at 12 (p=0.0030), 18 (p=0.023), and 24 (p=0.033) than it had been at baseline, but it increased to a similar proportion as baseline by month 30 (p=0.176; appendix);although, the overall prevalence of lesions remained much lower than baseline. The proportion of ulcers due to *H. ducreyi* remained relatively stable during the 42 months of follow up (appendix).

DISCUSSION

In our study, we found that one mass administration of antibiotics followed by targeted treatment of symptomatic cases and their contacts caused a transient sharp decline on infection levels and transmission, but did not eliminate yaws in a highly endemic island community. To our knowledge, this is the largest studythat evaluates a yaws eradication program with oral antibiotics and with a long, 42-month follow-up. The initial impact of a single-dose treatment with oral azithromycin was a large reduction in prevalence of active yaws, similar to the findings subsequently described for Ghana and Solomon Islands.[8-9] The maximum impact of the intervention in this study was observed by the end of the second year, but thereafter the overall prevalence of yaws increased with many children presenting active yaws lesions in all villages. When we did molecular typing of *T. p. pertenue* samples from Lihir, we found a great reduction in genetic diversity of the circulating strains over time, which reached a minimum at 24 months when only one genotype was present. A reduction in genetic diversity and in seroreactivity of children 1-5 years old indicated an overall reduction in transmission, and hence the susceptibility of the bacteria population to the MDA intervention.

The relapse of untreated latent infections was the most important factor that hindered elimination efforts in this community, along with, to a lesser extent, the reintroduction of yaws throughcases of in-migration. Almost half of the individuals with new infections at follow-up surveys had not been

present for MDA. Although we had not evaluated baseline serostatus for these children (because it was impractical and impossible for financial reasons to assess the baseline serrostatus of everyone on the entire island), given the high prevalence of seropositivity of about 30% in randomly selected children at baseline, the active yaws episode was likely to be due to relapsed latent yaws. This finding is significant because it highlights the importance of achieving high initial treatment coverage of all persons to be sure of treating latent cases. Results of studies done in the 1950s indicate that because of the tendency of latent yaws to relapse early in the course of untreated infection, it is critical to treat latent infections as part of eradication efforts.[19-21] In addition, results of a mathematical model predict that >65% coverage of latent cases is required during each total targeted treatment program to achieve eradication;[22] however, contact tracingis unlikely to detect this proportion of latently infected individuals. Given the high coverage requirements for latent cases, and the relatively high fixed-costs of reaching endemic communities when compared to the relatively low costs of generic azithromycin, doing multiple rounds of MDA before the switch to total targeted treatment may be prefereable. Determination of the optimal number of MDA rounds to achieve eradication, the best intervals between rounds, and suitable interventions to prevent repeated non-attendance at MDA will require careful examination.

Importantly, we report the first documented macrolide resistance in *T. p. pertenue* infections. Analysis of five clinical specimens from 208 samples tested during the 42 month period after MDA, showed a *T. p. pertenue* strain carrying the 23S rRNA A2059G point mutation. We speculate that the first case likely to have had a *de novo* drug resistance mutation; antibiotic pressure has been associated with selection of mutants in syphilis studies.[23] A direct epidemiological association was present among all patients who had macrolide-resistant yaws suggesting that all had been infected with a single macrolide-resistant strain resulting from direct transmission by the index case; hence there was local spread of the resistant clone. The selection of the resistance mutation is likely to be a result of the yaws eradication programme implementation because azithromycin was used in these communities (after MDA) only for targeted treatment of yaws and to treat urethritis and genital ulcer disease in adults. For the syphilis agent, *Treponema pallidum* subsp. *pallidum*, the increase in prevalence of macrolide resistant strains has been rapid up to 64-100% in some developed countries.[24-28] However, whether this increase in prevalence is due to *de novo* point mutations or spread of a resistant strain, or both, is unclear.[14] In syphilis, macrolide-resistance mutations have been found in many different molecular strain types, consistent with *de novo* mutation. The frequency of emergence of primary resistance is proposed to be low because *T. pallidum* has two copies of the 23s rRNA gene and mutations in both operons seem to be required;[29] in all five mutant samples from our study, the same mutation A2059G, was found in both copies of the 23S rRNA gene. However, *Treponema denticola* (a related spirochete) can have phenotypic resistance with a mutation in only a single allele.[30] The role of dissemination of a single resistant strain in increased prevalence is not well defined for syphilis, as network analyses have not been performed. For yaws, travel of individuals is restricted in endemic countries, and the risk of spread from one country to another may be minimal. Emergence of macrolide-resistant strains, however, might cause local outbreaks.

The strengths of this study are the use of PCR to conclusively diagnose active yaws and the use of genotyping to differentiate between indigenous and imported cases. Additionally, the clinical surveys involved the entire population of interest, which is important to accurately measure a reduction of infection levels to zero because of the focal nature of the disease. A limitation of this study is the sampling of ulcers for PCR testing used in the first two rounds. Our aim was to swab a systematic sample of all observed ulcers, but this approach was not fully implemented in all villages. However, we believe that any association that might exist between the probability that an ulcer was swabbed and the probability that an ulcer was PCR positive is slight enough that it would not compromise the conclusions of the paper. Another limitation is the smaller than anticipated sample

size in latent infection surveys. This might have reduced the power of our study to show definitively whether transmission of yaws had ceased (i.e. infection in children 1-5 years indicates recent infection). However, the finding of zero cases of high titre latent yaws in repeated surveys, together with results showing an overall reduced genetic diversity of strains causing active yaws, strongly indicates that transmission was largely interrupted.

The generalizability of our findings is subject to three factors. First, rural Papua New Guinea might differ from other high-endemic zones in important environmental or cultural characteristics; however most yaws is found in rural tropical settings. Second, the impact of importation of infection could be larger in communities that are geographically contiguous to neighbouring areas where the disease is also endemic than in isolated island communities. Third, if the high coverage we achieved in MDA and subsequent total targeted treatment programs cannot be attained, as might be the case outside a research setting, re-emergence of disease could happen more rapidly.

Our findings have substantial implications for the scalability of yaws eradication programs internationally and support the following adaptations to the current WHO strategy. First, a considerable effort to achieve coverage rates >90% should be the goal in the first round of treatment. Second, distribution of a second or third round of azithromycin at 6-12 months intervals might be of substantial benefit. Third, efforts to eradicate yaws should aim to treat much broader geographical areas, especially in regions with substantial migration. Finally, clinical and biological surveillance needs to immediately detect drug resistance through the strengthening of capacities of laboratory networks in endemic countries. Because the identified macrolide resistance mutations appear to cause no fitness disadvantage in *T. pallidum*, resistant strains would likely persist in communities even in the absence of antibiotic pressure. Therefore, communities where significant resistance is identified will need guidelines for clinical and operational management of macrolide resistant yaws with benzathine benzylpenicillin treatment to achieve cure and to avoid dissemination of resistant strains.

Contributors

OM, KA, QB and SAL conceived and designed the study with input from SVB and JW. OM, WH, AK, RP, HA, and CGB implemented the study, and gathered data and samples. SAL and CG were primarily responsible for microbiological and molecular studies. SAL designed microbiological laboratory techniques and supervised laboratory work at University ofWashington. AEB conducted the diversity analyses. SS did the statistical analyses. OM and SAL wrote the first draft of the report. All authors contributed to revisions and approved the final version.

Declaration of interests

We declare no competing interests.

Acknowledgements

We thank the village chairmen, elders, and villagers of Lihir Island for their willingness to be involved in the study; our field teams for efforts with study implementation; and the National Department of Health of Papua New Guinea for guidance and oversight of the trial and continued cooperation, Sergi Gavilán (Barcelona Institute for Global Health, Barcelona, Spain) for project management and general administrative support, Laia Bertran (Barcelona Institute for Global Health, Barcelona, Spain)for effective knowledge management, inter-institutional liaison and acquisition of funding and Eric Q Mooring (Harvard TH Chan School of Public Health, Boston, MA, USA) for general advice on sampling and study design questions.This study was funded by ISDIN and Newcrest Mining Limited (OM); the laboratory work was supported in part by the US Public Health Service National Institutes of Health grant R01AI42143 (SAL); and WHO provided generic azithromycin.

FIGURE LEGENDS

Figure 1. Characteristics of PCR-confirmed active yaws by epidemiological history, molecular type, and macrolide resistance mutation.

PCR-confirmed active yaws refers to samples with positive results in either *tpN47* (*tp0574*) or *tp0548*, and in which the pertenue subspecies was confirmed by TprL PCR amplicon size. *T p pertenue=Treponema pallidum* subspecies *pertenue*. MDA=mass drug administration.

*A systematic random sample of 90 ([13%] out of 690) clinically suspected yaws cases at baseline and 84 ([69%]out of 121) at 6 months were tested by PCR; we provide data on the characteristics of lesions that were PCR-positive among the subset of PCR-tested lesions (31 at baseline and 41 at 6-months). In surveys between months 12 and 42, we tested all clinically suspected lesions by PCR; therefore we provide data on the characteristics of all lesions detected for these timepoints.

†Not all *T. p. pertenue* positive samples could be fully typed for all three typing targets.

§ P<0.0001 for the estimate of the mean evolutionary diversity of *T. p. pertenue* isolates at 24-month survey compared with baseline.

¶ Data are the mean evolutionary diversity of *T. p. pertenue* isolates at each round.

‡Not all *T. p. pertenue* positive samples could be amplified for 23S rRNA by PCR.

Figure 2. Yaws lesions in a patient with treatment failure associated with macrolide-resistant *Treponema pallidum* subsp. *pertenue*.

(A) Primary lesion (red, moist 2·5 cm ulcer) on the left leg of an 11-year-old patient with yaws observed at the 30 months survey. Lesional swab PCR was positive for *T. p. pertenue* with wild-type 23S rRNA. (B) Secondary yaws papillomas (multiple nodules with yellow-colour granular surface) seen at 36 months survey. These lesions were PCR positive for *T. p. pertenue* with A2059G mutation in 23SrRNA. (C) Photomicrograph of skin biopsy of the larger papilloma lesion in Panel B with abundant spirochete organisms stained bright red by the *Treponema pallidum* immunohistochemical stain (×400 magnification).

Table 1: Prevalence of skin ulcers and active yaws

Time post MDA	People in census	People examined	All Clinically Suspected Lesions		Active Yaws Lesions*		Clinically Suspected Lesions with Positive Serological Findings	
			No. of cases (%)	Prevalence ratio (95%CI) †‡	No. of cases (%)	Prevalence ratio (95%CI) †‡	No. of cases (%)	Prevalence ratio (95%CI)†‡
Baseline	16,092	13,490 (84%)	690 (5·1)	1	238 (1·8) §	1	323 (2·4)	1
6 mo.	16,092	13,166 (82%)	121 (0·9)	0·18 (0·15; 0·22)	59 (0·4) ¶	0·25 (0·19; 0·34)	44 (0·3)	0·14 (0·10; 0·19)
12 mo.	17,339	13,204 (76%)	114 (0·9)	0·17 (0·14; 0·21)	19 (0·1) ‖	0·08 (0·05; 0·13)	34 (0·3)	0·11 (0·08; 0·15)
18 mo.	17,339	15,977 (92%)	88 (0·6)	0·11 (0·09; 0·13)	17 (0·1) ‖	0·06 (0·04; 0·10)	38 (0·2)	0·10 (0·07; 0·14)
24 mo.	17,555	11,792 (67%)	68 (0·6)	0·11 (0·09; 0·14)	13 (0·1) ‖	0·06 (0·04; 0·11)	24 (0·2)	0·09 (0·06; 0·13)
30 mo.	17,555	14,935 (85%)	120 (0·8)	0·16 (0·13; 0·19)	31 (0·2) ‖	0·12 (0·08; 0·17)	52 (0·3)	0·15 (0·11; 0·19)
36 mo.	18,836	14,765 (78%)	107 (0·7)	0·14 (0·12; 0.17)	36 (0·2) ‖	0·14 (0·10; 0·20)	53 (0·4)	0·15 (0·11; 0·20)
42 mo.	18,836	13,601 (72%)	107 (0·8)	0·15 (0·13; 0.19)	51 (0·4) ‖	0·21 (0·16; 0·29)	63 (0·5)	0·19 (0·15; 0·25)

*Active yaws refers to the estimated number of participants with lesional PCR positive results in either *tpN47* (*tp0574*) or *tp0548*, and in which the pertenue subspecies was confirmed by TprL PCR amplicon size.

†The prevalence ratio was calculated by means of the log-binomial model. The baseline prevalence is the reference value.

‡ P<0.0001 for the significance of the model overall.

§ At baseline, a random sample of 90 (out of 690) clinically suspected yaws lesions were tested by PCR; the number of active yaws cases in the entire population were estimated using the proportion of PCR-positive specimens among the subset of PCR-tested lesions (34·4% [31/90]) multiplied by the total number of clinically suspected yaws lesions detected (n 690).

¶ At 6 months, the same approach as taken for baseline was used to estimate the number of active yaws cases. The proportion of PCR-positive specimens (48·8% [41/84]) was multiplied by the total number of clinically suspected yaws lesions detected (n 121).

‖ In 12-month to 42-month surveys, we tested all clinically suspicious lesions by PCR; therefore we obtained a direct measurement of the number of active yaws cases in the entire population.

Baseline, 6, and 12 month data were previously published (Ref. 7) and are included here for comparison to later time points.

Table 2. Proportion of non-travelling vs travelling participants with yaws in the post-MDA period according to genotypes

	Genotypes of yaws strains							Not fully typed cases (n=31)	Total (n=208)
	JG8 (n=149)	SE7 (n=11)	TG8 (n=1)	TD6 (n=11)	TG6 (n=3)	SD6 (n=1)	JD8 (n=1)		
Non-travelling resident*	125 (83·9%)	2 (18·2%)	0 (0·0%)	11 (100%)	3 (100%)	0 (0·0%)	1 (100%)	26 (83·9%)	168 (80·8%)
Travelling or in-migrated participant†	24 (16·1%)	9 (81·8%)	1 (100%)	0 (0·0%)	0 (0·0%)	1 (100%)	0 (0·0%)	5 (16·1%)	40 (19.2%)

*All non-travelling residents either absent or present at Mass Drug Administration.

†Travel history was assessed by self-reported travel out of Lihir Island to a yaws endemic area in the preceding 6 months, regardless of compliance to MDA. History of in-migration was assessed by self-reported migration to Lihir Island in the preceding 6 months and verified by non-appearance at the previous year census, regardless of compliance to MDA.

Table 3. Prevalence of latent yaws in subgroups determined by age

Time (month)	Children aged 1 – 5 years					Children aged 6 – 15 years				
	No. of children tested	All Cases of Latent Yaws*		High-titre Latent Yaws†		No. of children tested	All Cases of Latent Yaws*		High-titre Latent Yaws†	
		Children (%)	Adjusted prevalence ratio (95%CI)‡§	Children (%)	Adjusted prevalence ratio (95%CI)‡¶		Children (%)	Adjusted prevalence ratio (95%CI)‡ ‖	Children (%)	Adjusted prevalence ratio (95%CI)‡**
Baseline	117	26 (22·2)	1	16 (13·7)	1	874	299 (34·2)	1	165 (18·9)	1
6 mo	77	10 (13·0)	0·58 (0·20; 1·74)	6 (7·8)	0·57 (0·15; 2·19)	797	251 (31·5)	0·92 (0·55; 1·53)	117 (14·7)	0·78 (0·40; 1·53)
12 mo	114	6 (5·3)	0·24 (0·09; 0·63)	1 (0·9)	0·06 (0·01; 0·48)	796	143 (18·0)	0·53 (0·35; 0·79)	58 (7·3)	0·39 (0·19; 0·77)
18 mo	81	9 (11·1)	0·50 (0·22; 1·12)	1 (1·2)	0·09 (0·02; 0·52)	462	129 (27·9)	0·82 (0·55; 1·22)	50 (10·8)	0·57 (0·34; 0·98)
24 mo	69	6 (8·7)	0·39 (0·17; 0·88)	1 (1·4)	0·11 (0·01; 0·75)	445	113 (25·4)	0·74 (0·48; 1·15)	24 (5·4)	0·29 (0·16; 0·52)
30 mo	65	4 (6·2)	0·28 (0·10; 0·79)	0 (0·0)	--	416	136 (32·7)	0·96 (0·62; 1·48)	33 (7·9)	0·42 (0·18; 0·97)
36 mo	66	3 (4·5)	0·20 (0·05; 0·80)	1 (1·5)	0·11 (0·02; 0·78)	470	130 (27·7)	0·81 (0·47; 1·40)	22 (4·7)	0·25 (0·08; 0·81)
42mo.	68	6 (8·8)	0·40 (0·17; 0·94)	0 (0·0)	--	422	84 (19·9)	0·58 (0·37; 0·91)	28 (6·6)	0·35 (0·20; 0·62)

* The analysis included all seropositive children with a reactive TPHA and RPR titre of at least 1:2.

† The analysis included children with a reactive TPHA and RPR titre of at least 1:16.

‡ The adjusted prevalence ratio was calculated with the use of the cluster option of a log-binomial regression model. The baseline prevalence is the reference value.

For the significance of the overall model § P = 0·0165, ¶ P = 0·0014, ‖ P = 0·0024, ** P = 0·0002.

Baseline, 6 and 12 month data were previously published (Ref. 7) and are included here for comparison to later time points

Extended data Table 1: Aetiology of skin ulcers prior to MDA of azithromycin and in subsequent rounds of targeted treatment *†

Time (month)	Participants tested for PCR	*Treponema pallidum* subsp. *pertenue* only detected	Both *T. p.* pertenue and *H. ducreyi* detected	*Haemophilus ducreyi* only detected	Negative for *T. pallidum* and *H. ducreyi*
	no.	no. of participants (%)			
Baseline	90*	19 (21·1)	12 (13·3)	42 (46·7)	17 (18·9)
6 mo.	84*	14 (16·7)	27 (32·1)	32 (38·1)	11 (13·1)
12 mo.	114	12 (10·5)	7 (6·1)	53 (46·5)	42 (36·8)
18 mo.	88	12 (13·6)	5 (5·7)	35 (39·8)	36 (40·9)
24 mo.	68	6 (8·8)	7 (10·3)	28 (41·2)	27 (39·7)
30 mo.	120	19 (15·8)	12 (10·0)	63 (52·5)	26 (21·7)
36 mo.	107	24 (22·4)	12 (11·2)	49 (45·8)	22 (20·6)
42mo.	107	37 (34·6)	14 (13·1)	30 (28·0)	26 (24·3)

* Data of PCR-confirmed yaws cases for baseline and 6 months represent a random sample of 90 and 84 ulcers that were tested by PCR from a total of 690 and 121 participants with skin ulcers detected, respectively. Data from 12 months to 42 months represent all ulcers detected.

† $P<0.0001$ by the chi-square test for the between-group comparison within each type of infection.

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