Comparison of annual versus twice-yearly mass azithromycin treatment for hyperendemic trachoma in Ethiopia: a cluster-randomised trial

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Summary

Background **In trachoma control programmes, azithromycin is distributed to treat the strains of chlamydia that cause ocular disease. We aimed to compare the effect of annual versus twice-yearly distribution of azithromycin on infection with these strains.**

Methods **We did a cluster-randomised trial in 24 subdistricts in northern Ethiopia, which we randomly assigned to receive annual or twice-yearly treatment for all residents of all ages. Random assignment was done with the RANDOM and SORT functions of Microsoft Excel. All individuals were offered their assigned treatment of a single, directly observed, oral dose of azithromycin. A 6 week course of topical 1% tetracycline ointment, applied twice daily to both eyes but not directly observed, was offered as an alternative to azithromycin in patients younger than 12 months, and in patients with self-reported pregnancy, with allergy, or who refused azithromycin. Our primary, prespecified outcome was the prevalence of ocular chlamydial infection in a random sample of children aged 0–9 years at baseline and every 6 months for a total of 42 months within sentinel villages. Our analysis was by intention to treat. This study is registered with ClinicalTrials.gov, number NCT00322972.**

Findings **Antibiotic coverage of children aged 1–9 years was greater than 80% (range 80·9 to 93·0) at all study visits. In the groups treated annually, the prevalence of infection in children aged 0–9 years was reduced from a mean 41·9% (95% CI 31·5 to 52·2) at baseline to 1·9% (0·3 to 3·5) at 42 months. In the groups treated twice yearly, the prevalence of infection was reduced from a mean 38·3% (29·0 to 47·6) at baseline to 3·2 % (0·0 to 6·5) at 42 months. The prevalence of ocular chlamydial infection in children aged 0–9 years in groups treated annually was not different from that of the groups treated twice yearly at 18, 30, and 42 months (pooled regression p>0·99, 95 % CI –0·06 to 0·06). The mean elimination time in the twice-yearly treatment group was 7·5 months earlier (2·3 to 17·3) than that of the annual group (p=0·10, Cox proportional hazards model).**

Interpretation **After 42 months of treatment, the prevalence of ocular infection with chlamydia was similar in the groups treated annually and twice yearly. However, elimination of infection might have been more rapid in the groups of villages that received treatment twice yearly.**

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Introduction

Azithromycin is the treatment of choice for ocular infection with *Chlamydia trachomatis*, and mass antibiotic treatment is an essential component of the WHO global elimination programme.[1,2] A single mass azithromycin distribution results in a substantial reduction in the prevalence of infection and has been shown to be effective in many studies.[3–7] In districts where prevalence of clinically active trachoma is 10% or greater in children aged 1–9 years, WHO recommends at least three annual mass treatments before reassessment.[8] Although there is some evidence that more frequent treatment might be necessary, particularly in severely affected regions,[4] it is unknown if twice-yearly mass treatments are better.[9–11] The WHO Global Elimination of Trachoma campaign hopes to eliminate blindness due to trachoma worldwide by 2020.[12] Since trachoma is a communicable disease and is generally treated at the community level, we chose a cluster-randomised design to assess azithromycin for ocular chlamydial infection in a hyperendemic region of Ethiopia. We seek to establish the most rational use of antibiotic and whether local elimination of chlamydial infection is a feasible goal in an entire community.

Methods

Participants

Between May, 2006, and November, 2009, we did a cluster-randomised clinical trial in northern Ethiopia. The country is divided into woredas (districts) and our study took place in the Goncha Siso Enese woreda of the Amhara region. A woreda is further divided into subkebeles, an Ethiopian geographical unit that has about 1400 individuals in five state teams (a state team is similar to a village). A state team typically consists of about 50 households with about 275 individuals. In the Trachoma Amelioration in Northern Amhara (TANA) trial, 72 subkebeles were randomly

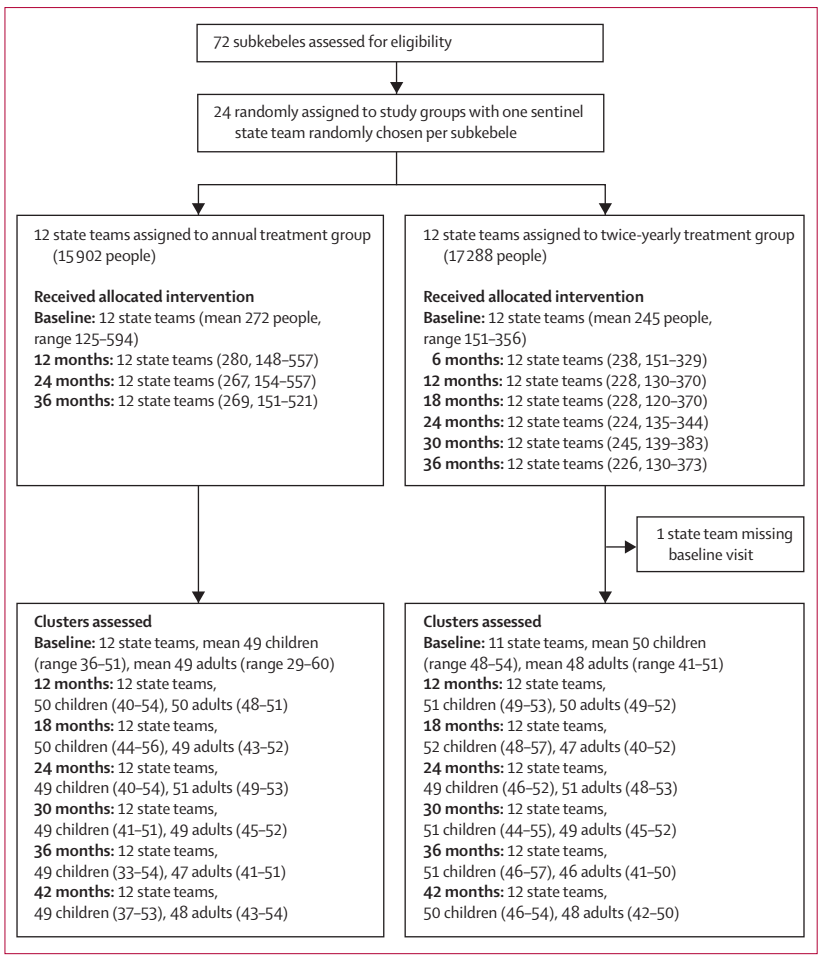

Figure 1: **Trial profile**

assigned to one of six groups of 12 subkebeles, allowing three separate trachoma-specific comparisons with 24 subkebeles in each comparison.[13,14] Eligible for inclusion were all subkebeles in the study region that were less than a 3 h walk beyond the furthest point that could be reached with a four-wheel drive vehicle. We report the results from 24 subkebeles that were randomly assigned (1:1) to receive annual treatment or twice-yearly treatment. The remaining 48 subkebeles from the original pool of 72 were entered into separate trials reported elsewhere.[13–15] All state teams within a subkebele received the same intervention in an effort to minimise contamination between subkebeles.

Informed consent from the parent or guardian was obtained (owing to the high rates of illiteracy in the region, fieldworkers read out the consent form and obtained verbal consent from the participants), as well as verbal agreement from children who were at least 7 years of age. Ethical approval for this study was obtained from the Committee for Human Research of the University of California, San Francisco (CA, USA); the Ethiopian Ministry of Science and Technology; and Emory University (Atlanta, Georgia). The study was done in accordance with the Declaration of Helsinki. A data and safety monitoring committee appointed by the National Institutes of Health–National Eye Institute oversaw the design and implementation of the study (NEI U10 EY016214).

Randomisation and masking

One sentinel state team was randomly selected from each of the 24 subkebeles for monitoring. In each of these sentinel state teams, 60 children and 60 adults were randomly selected and were requested to allow clinical assessment and conjunctival sampling for ocular infection with chlamydia. Selected individuals might not have been present because they had died, permanently moved, were temporarily absent, or refused. At each of the visits, the random sample was regenerated, so individuals might have been repeatedly chosen. Field examination teams were asked to make up to three return visits to state teams to increase their coverage at all timepoints. The randomisation sequence was done in three stages: six groups of 12 subkebeles each, one sentinel state team within each subkebele, and 60 children and 60 adults from each of the sentinel state teams at each visit. Randomisation of the subkebeles and state teams was done by KR with RANDOM and SORT functions in Microsoft Excel (version 2003) and for individuals by JH with the same software. All assignments were concealed until implementation. Censuses for all state teams were done by experienced and trained health-care personnel from whom treatment group and the prevalence of ocular chlamydial infection was masked. The baseline census included the name, age, and sex of each individual in each household. All households were assigned a number, and migration, deaths, and births were tracked at each subsequent census. Antibiotic coverage, treatment allocation, and all clinical trachoma and chlamydial infection outcomes were masked from census workers and data collection teams. All clinical trachoma and chlamydial infection outcomes were masked from antibiotic distributors. Treatment allocation and timepoint were masked from laboratory workers; however, they were aware of the relative time of the study as it progressed over the full 42 months. Treatment assignment was not masked from state team members.

Procedure

Conjunctival examination for clinically active trachoma and conjunctival swabbing for chlamydial PCR were done every 6 months.[9,13] Clinical grading of the right everted superior tarsal conjunctiva was done with the WHO simplified grading system.[16] Clinical graders were only allowed to grade for the trial if they had attained a sufficient chance corrected agreement ($\kappa \geq 0{\cdot}6$) with an experienced grader (BA, TML, or BDG) over the scoring of signs of clinically active trachoma (follicular inflammation, intense inflammation, or both, in the WHO system) in validation exercises in both the laboratory (photograph collection) and the field. We assessed grading agreement every 6 months throughout the trial. If κ dropped below 0·6 for

any grader, they received further training. After conjunctival examination, a Dacron swab was passed firmly three times over the right upper tarsal conjunctiva, rotating 120 degrees between each pass.[9,13] To assess field contamination at all visits, a negative field control passing within about 2·5 cm of, but not touching, the participant's conjunctiva was undertaken in five randomly selected children per state team, immediately after the initial study swab.[13] Field workers changed gloves before examining each new participant. All samples were immediately placed at 4°C in portable coolers containing ice packs, and then frozen at –20°C within 10 h. The swabs were shipped at 4°C to the University of California, San Francisco (CA, USA), where they were stored at –80°C until processing.[17] The Amplicor PCR assay (Roche Diagnostics, Branchburg, NJ, USA) was used to detect *C trachomatis* DNA and samples were pooled for processing to save time and cost, as previously described.[9,18] Briefly, pretreatment samples were tested in pools of two and post-treatment samples were randomly assigned, from the same state team, into groups of five. If the results from any pool were equivocal, then all samples from the pool were individually retested. We used maximum likelihood estimation to obtain the prevalence of ocular chlamydial infection in each state team on the basis of the number of positive samples most likely to have resulted in the measured pooled PCR result. This process saves time and cost but does not allow identification of individuals with positive PCR results without further testing.

All individuals were offered their assigned treatment of a single, directly observed, oral dose of azithromycin (height-based dosing equalling 20 mg/kg for children aged 1–15 years; 1 g for participants older than 15 years) in accordance with government and WHO guidelines.[6,10] A 6 week course of topical 1% tetracycline ointment (Shanghai General Pharmaceutical, Shanghai, China), applied twice daily to both eyes but not directly observed, was offered as an alternative to azithromycin in patients younger than 12 months, and in patients with self-reported pregnancy, with allergy, or who refused azithromycin. Within each subkebele, the intent was to reach the WHO target of at least 80% of the eligible population. Antibiotic coverage was estimated relative to the most recent, updated household-based census figures for all of the study communities. To assess the antibiotic component of the WHO trachoma elimination campaign as objectively as possible during the study, programmatic or study interventions to improve facial cleanliness or other environmental changes were not implemented.

Our primary, prespecified outcome was the prevalence of ocular chlamydial infection in children aged 0–9 years. We measured the prevalence of infection in state teams randomly assigned to receive annual treatments compared with state teams assigned to receive twice-yearly treatments at 18, 30, and 42 months after baseline. These monitoring visits were chosen a priori to minimise bias associated with the time since last treatment—at these three timepoints all state teams had received a treatment 6 months previously. All measurements were taken before treatment at all timepoints in both the state teams treated annually and twice yearly. We also compared the difference in prevalence of infection between the two groups at 12, 24, and 36 months as a prespecified secondary analysis.

Statistical analysis

We estimated that the inclusion of 12 subkebeles per treatment group (annual or twice yearly) would provide 80% power to detect a 6% difference in the prevalence of infection in individuals younger than 10 years, assuming an SD of 5% in the 12 month prevalence, a correlation between baseline and 12 months of 0·5, a two tailed α of 0·05, and a sample of 48 individuals per state team (of the 60 invited for examination). Our primary, prespecified analysis was pooled regression of the prevalence of infection at 18, 30, and 42 months. We tested the hypothesis that the coefficient corresponding to each group was not equal to zero, adjusting for the baseline prevalence and treatment time. We prespecified hypothesis tests to be two sided, with $\alpha=0\cdot05$. We used a square-root transformation of the outcome to improve normality and homoscedasticity. We computed approximate prediction intervals with the bootstrap percentile method. In an exploratory analysis, we included predictors individually to the regression model for the PCR prevalence in children at months 18, 30, and 42 in

	Annual treatment	Twice-yearly treatment
Number of state teams	12	12*
Total number of individuals per state team	320 (235–406)	286 (237–335)
Proportion of population aged 0–9 years	31·9% (29·1–34·8)	31·7% (29·2–34·2)
Proportion of female participants	48·2% (43·4–52·0)	48·7% (45·6–51·9)
Altitude (m)	2522 (2358–2687)	2593 (2455–2730)
Distance to closest town (km)	8·8 (6·8–10·9)	7·4 (5·4–9·3)
Prevalence of clinical trachoma†	68·7% (56·3–81·2)	76·9% (59·8–94·0)

Data are mean (95% CI). *Trachoma prevalence in 11 state teams with available data. †Defined as follicular inflammation, intense inflammation, or both by the WHO simplified grading scale.[16]

Table 1: **Baseline characteristics**

	Annual treatment group		Twice-yearly treatment group	
	Aged 1–9 years	Aged ≥10 years	Aged 1–9 years	Aged ≥10 years
Baseline	80·9% (72·4–89·4)	84·9% (76·6–93·1)	82·8% (79·1–86·4)	87·2% (83·7–90·8)
6 months	No treatment*	No treatment*	84·2% (81·7–86·7)	84·4% (82·1–86·8)
12 months	92·1% (88·9–95·3)	88·8% (85·1–92·5)	92·8% (89·6–96·0)	82·1% (73·9–90·3)
18 months	No treatment*	No treatment*	87·7% (82·8–92·6)	78·2% (70·2–86·2)
24 months	87·3% (79·9–94·8)	82·3% (73·7–90·9)	87·9% (83·4–92·3)	77·8% (68·9–86·9)
30 months	No treatment*	No treatment*	93·0% (88·9–97·1)	85·0% (78·1–91·9)
36 months	92·5% (87·1–98·0)	85·6% (77·9–95·3)	90·8% (85·9–95·7)	78·5% (71·7–85·3)

Data are mean (95% CI). Treatment with oral azithromycin (children 20 mg/kg; adults 1 g) or topical tetracycline. *No treatment offered at 6, 12, or 30 months as per study design.

Table 2: **Mean treatment coverage during mass antibiotic distributions by study visit**

addition to the treatment regimen. These predictors were altitude, distance to the nearest large regional centre (Gandewayn), proportion aged 0–9 years who were girls, proportion aged between 0 and 9 years, and accessibility. We gave each randomisation unit the same weight, and the prevalence in that cluster (the square-root transformed proportion) was used as a continuous outcome.

We did two-group comparisons of elimination times with a Cox proportional hazards model (which requires no distributional assumptions).[19] Elimination time was defined as the first of two consecutive study visits in which no chlamydial infection was detected in children aged 0–9 years. Because the prevalence of infection was unknown after 42 months, the 36 month timepoint was the last study visit at which elimination could happen for our study. We computed survival curves with the product-limit estimator, with approximate 95% CIs derived from the complementary log-log transformation and the Greenwood formula.[20] We did a survival analysis with the Cox proportional hazards model.[20,21]

Missing baseline prevalence data (which happened in one of 24 state teams because of a mistake in identifying the correct sentinel state team location) was handled according to a prespecified plan. For all state teams, including the state team with missing baseline data, the prevalence data at 6 months were available. In accordance with our analysis plan, we undertook multiple imputation of the missing baseline prevalence with the regression equation relating the 6 month prevalence to the baseline prevalence. As a secondary analysis, we also restricted the comparison to state teams for which complete data were available.

Our statistical analysis plan was proposed and approved before the availability of outcome data. Data were masked from the field workers and researchers until conclusion of all sample collections and were unmasked only after approval by the data and safety monitoring committee, as per our prespecified plan. No adjustments were made for missing individuals at any visit and all analyses were by intention to treat at the level of the state team. We did intention-to-treat analyses for all comparisons. We used the statistical package R for all analyses (version 2.12 for Macintosh). This study is registered with ClinicalTrials.gov, number NCT00322972.

	Baseline	6 months	12 months	18 months	24 months	30 months	36 months	42 months
Annual treatment								
1	44·9% (22/49)	28·0% (14/50)	38·0% (19/50)	7·5% (4/53)	12·8% (6/47)	6·0% (3/50)	12·2% (6/49)	1·9% (1/53)
2	46·0% (23/50)	30·2% (16/53)	8·0% (4/50)	8·0% (4/50)	12·2% (6/49)	0·0% (0/47)	8·2% (4/49)	8·9% (4/45)
3	53·1% (26/49)	10·9% (6/55)	18·5% (10/54)	4·0% (2/50)	3·7% (2/54)	0·0% (0/51)	0·0% (0/53)	0·0% (0/53)
4	52·0% (26/50)	7·5% (4/53)	15·7% (8/51)	1·8% (1/56)	3·9% (2/51)	0·0% (0/50)	0·0% (0/48)	2·0% (1/49)
5	56·0% (28/50)	8·0% (4/50)	19·2% (10/52)	2·0% (1/50)	8·0% (4/50)	4·0% (2/50)	2·0% (1/51)	2·1% (1/48)
6	14·0% (7/50)	2·0% (1/50)	4·0% (2/50)	2·0% (1/50)	2·0% (1/51)	2·0% (1/51)	2·0% (1/51)	2·0% (1/50)
7	16·7% (6/36)	4·9% (2/41)	5·0% (2/40)	2·3% (1/44)	2·5% (1/40)	0·0% (0/41)	0·0% (0/33)	0·0% (0/37)
8	36·0% (18/50)	2·0% (1/51)	0·0% (0/50)	0·0% (0/50)	0·0% (0/51)	0·0% (0/50)	2·1% (1/48)	0·0% (0/49)
9	58·0% (29/50)	18·5% (10/54)	32·7% (17/52)	5·8% (3/52)	6·0% (3/50)	2·0% (1/50)	11·5% (6/52)	3·9% (2/51)
10	22·4% (11/49)	26·5% (13/49)	20·0% (10/50)	0·0% (0/50)	0·0% (0/48)	0·0% (0/50)	0·0% (0/51)	0·0% (0/51)
11	62·0% (31/50)	7·5% (4/53)	5·9% (3/51)	4·3% (2/46)	4·5% (2/44)	0·0% (0/47)	0·0% (0/45)	0·0% (0/45)
12	41·2% (21/51)	24·0% (12/50)	8·0% (4/50)	15·7% (8/51)	4·0% (2/50)	6·0% (3/50)	3·7% (2/54)	2·0% (1/51)
Mean (95% CI)	41·9% (31·5–52·2)	14·2% (7·4–20·9)	14·5% (7·1–22·0)	4·5% (1·6–7·2)	5·0% (2·3–7·6)	1·7% (0·1–3·2)	3·5% (0·5–6·4)	1·9% (0·3–3·5)
Twice-yearly treatment								
13	32·0% (16/50)	5·6% (3/54)	5·8% (3/52)	0·0% (0/55)	0·0% (0/52)	0·0% (0/51)	0·0% (0/51)	2·0% (1/49)
14	50·8%*	36·5% (19/52)	50·0% (26/52)	20·0% (10/50)	12·0% (6/50)	9·1% (4/44)	7·0% (4/57)	15·7% (8/51)
15	46·9% (23/49)	16·7% (9/54)	3·9% (2/51)	1·9% (1/54)	0·0% (0/50)	0·0% (0/53)	0·0% (0/47)	0·0% (0/53)
16	26·0% (13/50)	7·8% (4/51)	8·2% (4/49)	4·2% (2/48)	4·2% (2/48)	4·0% (2/50)	4·0% (2/50)	2·0% (1/49)
17	8·2% (4/49)	2·0% (1/51)	2·0% (1/51)	2·0% (1/49)	0·0% (0/50)	0·0% (0/51)	0·0% (0/50)	0·0% (0/48)
18	58·0% (29/50)	8·0% (4/50)	0·0% (0/51)	0·0% (0/50)	0·0% (0/50)	0·0% (0/55)	0·0% (0/52)	3·7% (2/54)
19	47·9% (23/48)	54·9% (28/51)	26·0% (13/50)	8·3% (4/48)	4·1% (2/49)	2·0% (1/50)	1·9% (1/52)	1·9% (1/52)
20	46·0% (23/50)	8·0% (4/50)	2·0% (1/49)	4·0% (2/50)	2·0% (1/49)	1·9% (1/54)	0·0% (0/52)	0·0% (0/50)
21	30·0% (15/50)	0·0% (0/50)	0·0% (0/53)	0·0% (0/56)	0·0% (0/49)	0·0% (0/49)	0·0% (0/46)	0·0% (0/46)
22	48·1% (26/54)	5·6% (3/54)	5·8% (3/52)	2·0% (1/49)	0·0% (0/50)	0·0% (0/52)	6·0% (3/50)	2·0% (1/50)
23	38·0% (19/50)	12·2% (6/49)	5·9% (3/51)	7·4% (4/54)	4·0% (2/50)	0·0% (0/51)	0·0% (0/50)	11·5% (6/52)
24	23·5% (12/51)	4·0% (2/50)	0·0% (0/51)	0·0% (0/57)	0·0% (0/46)	0·0% (0/49)	1·9% (1/52)	0·0% (0/50)
Mean (95% CI)	38·3% (29·0–47·6)	13·4% (3·2–23·7)	9·1% (2·6–18·1)	4·2% (0·5–7·8)	2·2% (0·7–4·4)	1·4% (0·2–3·1)	1·7% (0·6–6·4)	3·2% (0·0–6·5)

*Missing baseline data from a single state team were imputed, as per our prespecified analysis plan.

Table 3: **Estimated prevalence of ocular chlamydial infection in children aged 0–9 years by state team**

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Role of the funding source
The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Figure 1 shows the trial profile. Enrolment and recruitment began in June, 2006, and continued until the final treatment and follow-up in November, 2009. The baseline demographic and clinical characteristics of the two treatment groups were similar with respect to the proportion of girls, proportion aged 0–9 years, altitude of the state team, distance to the nearest population centre, and baseline trachoma clinical activity (table 1). 15 902 people from 12 subkebeles (all ages) were randomly assigned annual treatment and 17 288 people from 12 subkebeles were randomly assigned twice-yearly treatment. All subkebeles and sentinel state teams received their assigned treatment and none were lost to follow-up. Over the course of our study, 52 131 antibiotic treatments (50 048 azithromycin [96%] and 2083 tetracycline [4%]) were given annually, and 97 552 (94 112 azithromycin [96%] and 3440 tetracycline [4%]) were given twice yearly, to children and adults in state teams. Of 367 treatment refusals in both children and adults, 187 were in the state teams assigned annual treatment and 189 were in those assigned twice-yearly treatment. The three main reasons for treatment refusals were fasting (some orthodox Christians), farming and harvesting, or a previous adverse event in the individual who refused or in a family member.

Antibiotic treatment coverage of children aged 1–9 years was greater than 80% at all study visits (table 2). Treatment with oral drugs was directly observed; however, we were unable to establish the adherence to topical therapy over the full 6 week course after the medicine was distributed, because it was not directly observed. There were no serious adverse events attributable to the study drug reported through a passive surveillance system throughout the entire length of our study. We did a detailed adverse event survey after the distribution of antibiotics at 12 months (118 households) and 24 months (119 households) in a

	Baseline	6 months	12 months	18 months	24 months	30 months	36 months	42 months
Annual treatment								
1	8·0% (4/50)	0·0% (0/47)	7·8% (4/51)	0·0% (0/50)	1·9% (1/53)	2·0% (1/49)	0·0% (0/46)	2·2% (1/45)
2	16·0% (8/50)	4·1% (2/49)	6·0% (3/50)	2·1% (1/47)	2·0% (1/51)	0·0% (0/51)	6·0% (3/50)	0·0% (0/45)
3	22·0% (11/50)	3·9% (2/51)	8·0% (4/50)	0·0% (0/50)	0·0% (0/51)	0·0% (0/48)	0·0% (0/46)	0·0% (0/43)
4	16·0% (8/50)	4·1% (2/49)	2·0% (1/50)	0·0% (0/43)	0·0% (0/50)	0·0% (0/52)	0·0% (0/50)	0·0% (0/48)
5	26·0% (13/50)	3·7% (2/54)	6·0% (3/50)	2·0% (1/50)	3·9% (2/51)	0·0% (0/47)	2·0% (1/49)	1·9% (1/54)
6	6·7% (3/45)	2·0% (1/50)	4·0% (2/50)	0·0% (0/49)	0·0% (0/50)	2·0% (1/49)	0·0% (0/41)	0·0% (0/50)
7	5·0% (3/60)	4·1% (2/49)	6·0% (3/50)	2·0% (1/49)	4·0% (2/50)	0·0% (0/51)	0·0% (0/51)	0·0% (0/44)
8	6·0% (3/50)	2·0% (1/50)	2·1% (1/48)	0·0% (0/50)	2·0% (1/49)	0·0% (0/49)	2·1% (1/47)	0·0% (0/51)
9	26·0% (13/50)	16·3% (8/49)	20·0% (10/50)	6·1% (3/49)	7·8% (4/51)	0·0% (0/50)	2·4% (1/42)	0·0% (0/50)
10	6·9% (2/29)	1·9% (1/52)	4·0% (2/50)	0·0% (0/50)	2·0% (1/50)	0·0% (0/50)	0·0% (0/50)	0·0% (0/49)
11	16·0% (8/50)	2·2% (1/46)	0·0% (0/50)	0·0% (0/52)	0·0% (0/52)	0·0% (0/45)	5·9% (3/51)	0·0% (0/46)
12	4·1% (2/49)	0·0% (0/50)	8·0% (4/50)	0·0% (0/50)	4·1% (2/49)	0·0% (0/50)	0·0% (0/41)	0·0% (0/51)
Mean (95% CI)	13·2% (8·0–18·4)	3·7% (0·1–6·4)	6·2% (2·9–9·4)	1·0% (0·2–2·1)	2·3% (0·8–3·8)	3·4% (0·0–0·9)	1·5% (0·1–3·0)	0·3% (0·0–0·8)
Twice-yearly treatment								
13	12·2% (6/49)	2·0% (1/49)	0·0% (0/50)	0·0% (0/47)	2·0% (1/51)	0·0% (0/45)	0·0% (0/48)	0·0% (0/49)
14	16·0%*	6·3% (3/48)	4·1% (2/49)	12·5% (5/40)	6·0% (3/50)	1·9% (1/52)	2·4% (1/42)	0·0% (0/49)
15	14·6% (6/41)	8·2% (4/49)	1·9% (1/52)	2·0% (1/50)	0·0% (0/50)	0·0% (0/49)	0·0% (0/44)	0·0% (0/47)
16	16·0% (8/50)	8·2% (4/49)	2·0% (1/51)	1·9% (1/52)	0·0% (0/53)	0·0% (0/50)	0·0% (0/49)	0·0% (0/50)
17	2·0% (1/50)	2·3% (1/43)	2·0% (1/50)	0·0% (0/51)	0·0% (0/48)	0·0% (0/49)	0·0% (0/50)	0·0% (0/47)
18	26·5% (13/49)	0·0% (0/50)	2·0% (1/50)	0·0% (0/51)	4·0% (2/50)	0·0% (0/50)	0·0% (0/45)	0·0% (0/48)
19	28·0% (14/50)	4·3% (2/46)	0·0% (0/50)	2·0% (1/50)	3·9% (2/51)	2·0% (1/50)	0·0% (0/49)	0·0% (0/50)
20	25·0% (11/44)	2·0% (1/50)	2·0% (1/51)	0·0% (0/47)	0·0% (0/50)	0·0% (0/50)	0·0% (0/46)	0·0% (0/50)
21	8·0% (4/50)	2·0% (1/50)	0·0% (0/51)	0·0% (0/43)	0·0% (0/53)	0·0% (0/47)	0·0% (0/49)	0·0% (0/49)
22	21·7% (10/46)	2·0% (1/51)	0·0% (0/50)	2·2% (1/46)	0·0% (0/50)	0·0% (0/50)	0·0% (0/43)	0·0% (0/45)
23	13·7% (7/51)	0·0% (0/50)	2·0% (1/50)	0·0% (0/50)	0·0% (0/50)	2·2% (1/46)	0·0% (0/41)	4·8% (2/42)
24	8·9% (4/45)	6·0% (3/50)	4·1% (2/49)	2·4% (1/42)	2·0% (1/50)	2·0% (1/51)	0·0% (0/45)	0·0% (0/48)
Mean (95% CI)	16·1% (10·4–21·7)	3·6% (1·8–5·4)	1·7% (0·7–2·6)	1·9% (0·5–4·0)	1·5% (0·2–2·8)	6·7% (0·0–1·3)	0·2% (0·0–0·6)	0·4% (0·0–1·2)

*Missing baseline data from a single state team were imputed, as per our prespecified analysis plan.

Table 4: **Estimated prevalence of ocular chlamydial infection in people aged 10 years and older by state teams**

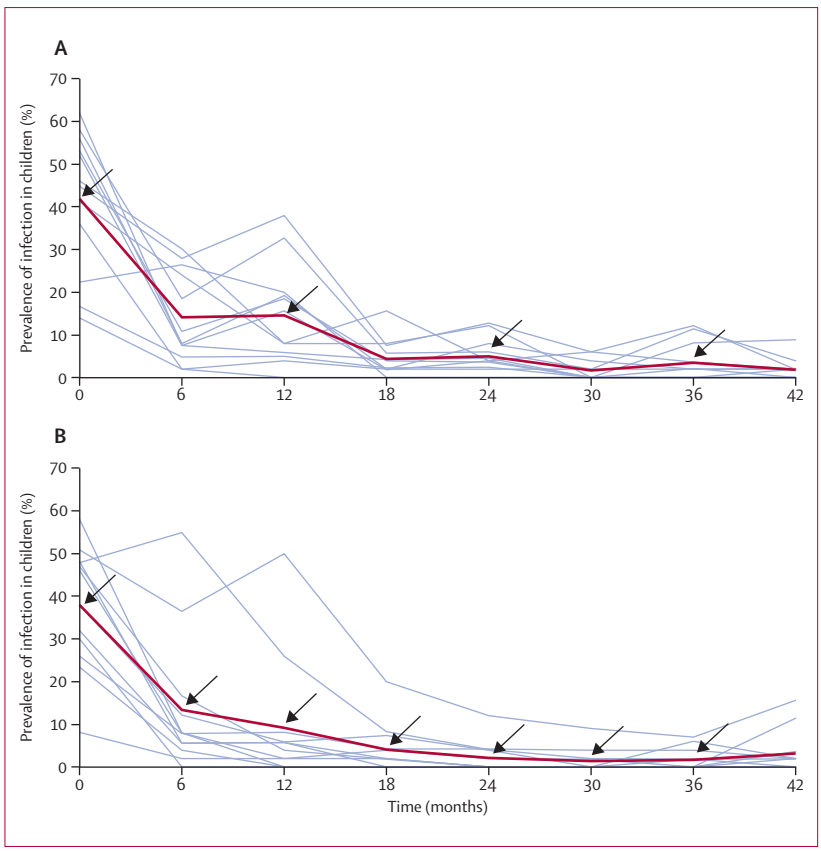

Figure 2: **Prevalence of ocular chlamydial infection in children aged 0–9 years**
In communities randomly assigned to annual (A) or twice-yearly (B) treatment. The arrows represent mass treatments with azithromycin. Missing baseline data from a single state team were imputed, as per our prespecified analysis plan.

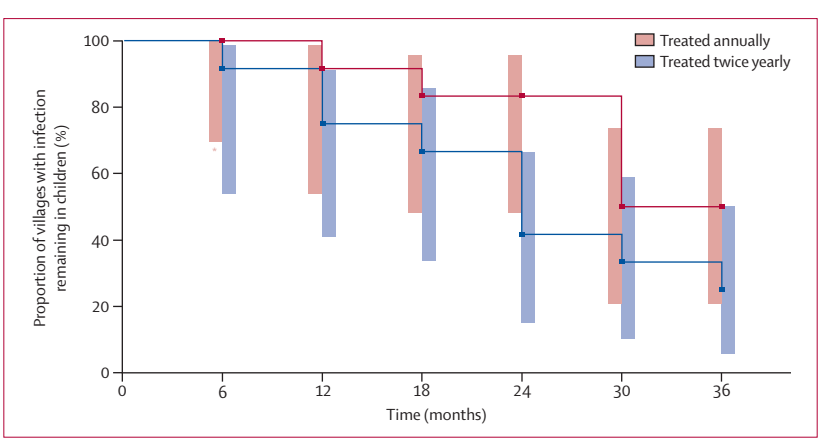

Figure 3: **Estimated proportion of state teams in which infection remains**
Annual and twice-yearly observations were made simultaneously, but are separated on the horizontal axis for clarity. Shaded bars show the 95% CI. We defined elimination as at least two consecutive state team visits with zero infections with *Chlamydia trachomatis* detected by PCR in children aged 0–9 years and is graphed at the first of these two treatment times. *Because no villages in the group treated annually showed elimination at 6 months, we used a one-sided 97·5% CI for this annual observation.

random sample from 24 state teams.[22] The prevalence of any adverse event ranged from 4·9% to 7·0% in children aged 1–9 years and 17·0% to 18·7% in people aged 10 years or older; the most common adverse events were abdominal pain (0·7% to 6·7%) and vomiting (1·4% to 4·5%). A single negative field control tested positive by PCR at the baseline visit and all subsequent field controls, done at all visits, were negative (one [0·1%] of 945).

The estimated prevalence of infection is shown in children (table 3) and adults (table 4) in treated state teams at all timepoints (figure 2). We compared infection elimination time in children aged 0–9 years between the annual and twice-yearly treatment groups (our primary prespecified analysis), adjusting for baseline prevalence (figure 3). Twice-yearly treatment was associated with a relative hazard for elimination of 2·4 (95% CI 0·85 to 6·98, p=0·10, Cox proportional hazards model) consistent with a shorter infection elimination time. Elimination was achieved in six (50%) of the 12 state teams treated annually and nine (75%) of the 12 state teams treated twice yearly by the 36 month visit. Pooled regression with PCR-based prevalence of infection in children aged 0–9 years at 18, 30, and 42 months (our primary prespecified timepoints) revealed no evidence of a difference between state teams treated annually or twice yearly (p>0·99, 95% CI –0·06 to 0·06, with multiple imputation for the state team with missing baseline prevalence). Altering the method of imputation or regression did not change the results. At baseline, the single missing state team had 290 individuals (81 children aged 0–9 years, 209 adults), was at an altitude of 2486 m, the distance to the closest town was 11·1 km, and the average antibiotic coverage over the full course of the study was 90·5% in children and 78·9% in adults. Complete case analysis (excluding the state team with missing baseline prevalence) yielded no evidence of a difference in prevalence between the two groups (p=0·33), and no value of the single missing baseline prevalence changed our conclusions. However, when assessing the prevalence of infection at 12, 24, and 36 months, we identified that the prevalence in children was higher in the communities treated annually (pooled regression, p=0·04, prespecified secondary comparison). The groups treated annually had an estimated infection prevalence 2·2% greater (95% CI 0·76 to 4·10) at 36 months (assuming a state team with the average baseline prevalence). Complete case analysis (omitting the state team with missing baseline prevalence) also yielded significant results (p=0·0004).

We did not identify a difference in PCR-based prevalence between the two treatment groups, in people aged 10 years and older (prespecified secondary analysis), at 18, 30, and 42 months (p=0·48, pooled regression). However, at 12, 24, and 36 months, the group treated twice yearly had a lower prevalence of infection in people aged 10 years or older (p=0·004, pooled regression). Aggregating over all visits, the prevalence of infection in adults was correlated with that in children (Spearman r=0·53, 95% CI 0·41 to 0·64).

We noted improvements in clinically active trachoma (a secondary analysis) in children aged 0–9 years from a prevalence of 68·7% (95% CI 56·3 to 84·2) at baseline to 31·5% (21·6 to 41·3) at 42 months in the state teams

treated annually and from 83·9% (75·7 to 92·1) at baseline to 35·0% (23·9 to 46·1) at 42 months in the state teams treated twice yearly. We were unable, with pooled regression, to detect a difference in clinically active trachoma between the state teams treated annually and twice yearly at 18, 30, and 42 months in children aged 0–9 years (p=0·12) or in people aged 10 years or older (p=0·35). Likewise, at 12, 24, and 36 months, with pooled regression, we were unable to detect a difference in clinically active trachoma between the state teams treated annually and twice yearly in children aged 0–9 years (p=0·70) or in people aged 10 years or older (p=0·17).

In exploratory analyses, we did not identify a difference in infection between treatment groups after adjusting for altitude (p=0.28), distance to the regional centre of Gandewayn (p=0·38), proportion of children who are girls aged 0–9 years (p=0·04, not significant after Holm adjustment for multiple comparisons), proportion of children aged 0–9 years (p=0·22), and accessibility (p=0·34). Furthermore, we did not identify evidence of effects of these additional predictors, except that the proportion of girls was associated with higher infection prevalences (p=0·0002).

Discussion

Our findings show a substantial reduction in chlamydial infection in both children and adults, irrespective of whether they were treated annually or twice yearly. 15 (63%) of 24 state teams received at least two consecutive visits in our trial where ocular chlamydial infection was not detected in any of the monitored children aged 0–9 years. Therefore, it seems that elimination is a feasible goal in severely affected communities with either annual or twice-yearly mass treatments. Elimination time was 7·5 months earlier in the twice-yearly treated group. It is unclear what the long-term implications are of this difference in elimination time; however, it is conceivable that communities that eliminate infection more quickly will have less burden of blinding eye disease over the long term. Although our results are encouraging, cautious interpretation is advised, because infection fell to 1·7 % at 36 months and then rose to 3·2% at 42 months on average in the state teams treated twice yearly. At the final 42 month surveillance, elimination had been previously achieved but infection subsequently returned in one annually treated and four twice-yearly treated state teams. As with other previous trachoma trials, there is a large variance in the estimated prevalence of infection between communities and even within the same community over time.[23] The mean infection in these 12 state teams behaves as we would expect (for example, decreasing steadily with distributions and returning to some extent without treatment), but this pattern is not necessarily seen in any particular state team. Apparent re-emergence at the conclusion of our study, after the final treatment, underscores the difficulty in maintaining elimination in communities where elimination might already have been achieved.[24] This finding might be caused by false-negative laboratory testing, sampling of only a portion of the state team rather than all individuals within the state team, contact with neighbouring state teams where infection is still present, or by migration.[4,7,25] It is also possible that there was contamination between the two groups of our study; however, we did not undertake specific measurements of movement between our two study groups. After mass treatment, infection is more difficult to detect, particularly because we were sampling only a subset of the entire state team. It is possible that a more sensitive assay (RNA-based PCR) would have detected infection in regions where we were unable to by use of the Amplicor DNA-based assay. We did not do a detailed analysis of travel, migration, or off-study drug use and this might be an important measurement for the future as we attempt to achieve and maintain favourable outcomes. Adjunctive measures such as environmental improvements might also be crucial in maintaining low levels of infection once achieved; however, there were no adjunctive measures implemented as part of our study of mass azithromycin treatment in an effort to measure the antibiotic effect more precisely.

In a previous study[9] in a different region of Ethiopia with hyperendemic trachoma, we identified that 2 years of twice-yearly mass azithromycin treatments reduced the prevalence of ocular chlamydia more than annual

Panel: Research in context

Systematic analysis

We present information on community-level mass antibiotic treatment based on the WHO's Alliance for the Global Elimination of Trachoma (GET) recommendations.[8] Studies have shown that azithromycin is effective for control of trachoma, and that mass azithromycin distributions are effective for control of ocular chlamydial infection. Case reports from twice-yearly treated communities have shown elimination of infection and a single randomised clinical trial suggested that twice-yearly treatment would be better than annual treatment for elimination, at least by 24 months.[9] We searched PubMed up to June 28, 2011, with the MeSH headings "*Chlamydia trachomatis*", "administration, oral", "administration, topical", "anti-bacterial agents", "azithromycin", "randomized controlled trials", "tetracycline", "biannual", and "trachoma". Our search was not restricted by language, year of publication, or study quality. We also assessed the Cochrane review "Antibiotics for Trachoma" of Dec 11, 2010 (updated Feb 18, 2011).[26] Our search of PubMed and the Cochrane review identified nine cluster-randomised clinical trials on mass antibiotics for trachoma or ocular chlamydial infection. Only four of these trials were both randomised and assessed at the community,[3,9,13,15,27] rather than individual, level and our study will be the fifth. Our study has the longest duration (3·5 years) of any cluster-randomised, cluster-analysed trial for trachoma or ocular chlamydial infection published so far.

Interpretation

We show that twice-yearly mass antibiotic administration might not be superior to annual administration, even in regions hyperendemic for chlamydial infection, over a 3·5 year period. One important aspect of our study is that when infection is measured at 18, 30, and 42 months after treatment (our primary prespecified outcome) we were unable to detect a difference in infection prevalence in the annual and twice-yearly treated communities. This finding has implications for trachoma treatment programmes worldwide and for the WHO global elimination goal.

treatments (panel). Our present study is consistent with this result and showed a similar effect at 12, 24, and 36 months with pooled regression. The timing of the treatment and measurement is crucial when interpreting these findings. The prevalence of ocular chlamydia at 12, 24, and 36 months could be lower in the twice-yearly group simply because state teams had been treated more recently. At 18, 30, and 42 months, all state teams had been treated 6 months before measurement and we did not identify a difference in infection at these times. This supports the notion that annual and twice-yearly treatment regimens are similar when measured at these timepoints, although the twice-yearly group had received more treatments. Another distinction from our previous study is that we designed our present trial to test whether repeated mass azithromycin treatments given for an even longer period would result in elimination of chlamydial infection, and whether twice-yearly mass treatments remain superior to annual mass treatments when given over this 3·5 year period.

The clinical examination for trachoma is poorly correlated with laboratory testing in other studies[28–30] because it is difficult to mask treatment from examiners and reproducibility is poor, but it remains the standard for many programmes and the WHO trachoma elimination campaign.[8] We identified a reduction in clinically active trachoma in some state teams between the baseline measurement and 42 months in both treatment groups (data not shown). We were unable to show that this reduction was significantly different between the annual and twice-yearly treated state teams with pooled regression at 18, 30, and 42 months or at 12, 24, and 36 months. Furthermore, we expect the signs of clinically active trachoma to resolve slowly in hyperendemic regions, similar to our present study, even after successful reduction of ocular chlamydia with mass treatments.[31,32]

There are substantial costs to any mass-treatment programme, including drug and distribution cost, increasing adverse events, and potential emerging drug resistance. Although macrolide resistance in *C trachomatis* has not been reported, antibiotic resistance has been reported in nasopharyngeal pneumococcus associated with mass treatment programmes.[33–36] In 12 neighbouring subkebeles, randomly assigned from the same pool of subkebeles in the Trachoma Amelioration in Northern Amhara study to receive mass azithromycin treatment of children aged 1–10 years at months 0, 3, 6, and 9,[13] mean prevalence of azithromycin resistance increased from 3·6% (95% CI 0·8 to 8·9) at baseline to 46·9% (38·5 to 57·5) at 12 months (p=0·003).[34] However, in a setting elsewhere in Ethiopia, emerging drug resistance decreased after cessation of a programme of mass azithromycin treatment for trachoma from a peak of 77% after six twice-yearly mass treatments to 21% 2 years after the final treatment.[37] The effect of increasing macrolide resistance on overall morbidity and mortality in communities that are part of a programme of mass antibiotic distribution is unknown, but cases of invasive pneumonia caused by resistant pneumococcus have not been associated with worse outcomes.[38] A reasonable conclusion is that programmes that use fewer doses of antibiotic drugs to obtain a similar therapeutic result for ocular chlamydial infection would be superior because of lower cost and less selective pressure for resistance.

The generalisability of our findings is limited by three factors. First, rural Ethiopia, of substantial importance as a zone of trachoma hyperendemicity, might nonetheless differ from other hyperendemic zones in important environmental or cultural characteristics. Second, the presence of decreasing infection in the absence of a control programme (secular trend) could amplify the apparent effect of a control programme. To the extent that the substantial (and expected) drops in infection prevalence we recorded were partly attributable to effects other than antibiotic distribution, our results would overestimate the decrease in prevalence after treatment when applied to a region in which no such secular trend was present. Such trends have been reported in other settings,[39–42] although we did not note a secular trend in 12 untreated neighbouring control state teams during the first 12 months of our study. The prevalence of infection in these untreated state teams at 12 months was 45·6 % (95% CI 36·7 to 54·5), similar to that identified in our study state teams at baseline.[13] These control state teams were then given mass azithromycin treatment for programmatic reasons, so we were unable to completely exclude the possibility that there was a secular trend from 12 to 36 months. All the state teams had been treated by the end of our study, precluding our ability to measure any trends over the full duration of our trial. But a drop in chlamydial infection from greater than 40% in children at baseline to about 3% at 42 months does not seem plausible to ascribe solely to a secular trend. And third, our results might be less applicable when the high antibiotic coverage we achieved (≥80%) cannot be achieved, as might be the case outside a research setting.

In summary, we have shown that the prevalence of ocular infection with chlamydia can be reduced to zero in a hyperendemic region in a random sample of children within a state team treated with two different strategies. This reduction was accomplished without implementation of adjunctive programmatic measures that used high antibiotic treatment coverage. To sustain elimination and prevent reintroduction of infection, adjunctive measures might be needed. If these measures prove to be as effective as hoped, elimination might be easier to achieve and to sustain.

Contributors

NES, JIH, PME, JDK, TCP, TML, and BDG came up with the concept and designed the study. TG, BA, MZ, AG, NES, JIH, JDK, TML, and BDG did the data acquisition. ZZ, JDK, TCP, TML, and BDG did the data analysis. JDK, TCP, TML, and BDG drafted the report. TG, ZZ, PME, JDK, TCP, TML, and BDG critically revised the report for intellectual content. TG, BA, NES, JIH, JDK, TML, and BDG supervised the study. ZZ, TCP, TML, and BDG did the statistical analysis. PME, JDK, TML, and BDG obtained the funding. TG, BA, MZ, AG, NES, ZZ, JIH, SY, KJR, PME, JDK, TML, BDG provided administrative and technical support.

Conflicts of interest

We declare that we have no conflicts of interest.

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