Effect of a Single Mass Antibiotic Distribution on the Prevalence of Infectious Trachoma

Jaya D. Chidambaram, MBBS
Wondu Alemayehu, MD, MPH
Muluken Melese, MD, MPH
Takele Lakew, MD, MPH
Elizabeth Yi, BA
Jenafir House, MPH, MSW
Vicky Cevallos, MT
Zhaoxia Zhou, BS
Kathryn Maxey, MS
David C. Lee, BA
Brett L. Shapiro, BA
Muthiah Srinivasan, MBBS
Travis Porco, PhD, MPH
John P. Whitcher, MD, MPH
Bruce D. Gaynor, MD
Thomas M. Lietman, MD

Context The World Health Organization recommends mass antibiotic distributions in its strategy to eliminate blinding trachoma as a public health concern. Some hypothesize that a single distribution is sufficient to control the ocular strains of chlamydia that cause trachoma. Others believe infection will inevitably return and periodic treatments or other measures are essential.

Objective To determine whether ocular chlamydial infection returns to the community up to 24 months after a single mass antibiotic distribution in a hyperendemic region of Ethiopia.

Design, Setting, and Participants Longitudinal cohort study conducted March 2003 to March 2005 in the Gurage Zone of Ethiopia. Eight randomly selected villages were assessed for ocular chlamydial infection. Fifteen untreated villages were randomly chosen at 12 months to allow assessment of a secular trend.

Intervention A single dose of oral azithromycin was offered to all residents of the 8 selected villages who were aged 1 year or older.

Main Outcome Measure Prevalence of ocular chlamydial infection in all children aged 1 to 5 years from each intervention village prior to treatment and 2, 6, 12, 18, and 24 months after mass antibiotic treatment, and also in untreated villages enrolled at 12 months.

Results Five hundred fifteen children were examined for ocular chlamydial infection at baseline. For the follow-up examinations, the mean participation rate was 83%. The mean prevalence of infection in children aged 1 to 5 years decreased from 43.5% (95% confidence interval [CI], 35.0%-52.0%) to 5.1% (95% CI, 1.1%-9.2%) after treatment. On average, infection returned gradually over 24 months to 11.3% (95% CI, 4.5%-18.1%; $P=.001$). In 7 of 8 villages, infection was higher at 24 months than at 2 months. In the remaining village, no infection could be identified at any point after treatment. Villages enrolled at 12 months had significantly fewer infections than those enrolled 12 months earlier, suggesting a secular trend ($P<.001$).

Conclusions Ocular chlamydial infection was not eliminated in children aged 1 to 5 years after a single mass azithromycin distribution; it slowly returned over 24 months, although not to baseline levels. Repeated treatments or other effective measures will be necessary for elimination.

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AT PRESENT, TRACHOMA REmains the most important infectious cause of blindness in the world.[1] Repeated infection with the ocular strains of *Chlamydia trachomatis* can bring about scarring of the conjunctiva, resulting in a cascade of entropion, inward-turned eyelashes, and eventually blindness due to corneal opacity.[2] To reduce infection, the World Health Organization (WHO) has recommended community-wide antibiotic distributions as part of its strategy to eliminate blinding trachoma as a public health concern by the year 2020.[3] Previous studies have shown that in the short term, mass antibiotic distribution can dramatically decrease the prevalence of ocular strains of chlamydia in villages.[4-7]

In theory, repeated distributions can progressively reduce infection, as long as the coverage and frequency of treatment are high enough.[5,8] In practice, repeated treatments have come close to eliminating infection, at least in areas with moderate amounts of trachoma.[6,7] Current WHO guidelines recommend 3 annual mass distributions.[3] But is repeat treatment necessary? It has been suggested that infection may never return after a single mass treatment with high coverage.[6,9-13] This has been difficult to test because of the need to monitor a large number of villages

Author Affiliations: F. I. Proctor Foundation (Drs Chidambaram, Porco, Whitcher, Gaynor, and Lietman, Mss Yi, House, Cevallos, Zhou, and Maxey, and Messrs Lee and Shapiro), Departments of Ophthalmology (Drs Whitcher, Gaynor, and Lietman) and Epidemiology and Biostatistics (Drs Whitcher and Lietman), and Institute for Global Health (Drs Whitcher and Lietman), University of California, San Francisco; Orbis International, Addis Ababa, Ethiopia (Drs Alemayehu, Melese, and Lakew); Aravind Eye Hospital, Madurai, India (Dr Srinivasan); and California Department of Health Services, Berkeley (Dr Porco).
Corresponding Author: Thomas M. Lietman, MD, F. I. Proctor Foundation, Box 0412, 513 Parnassus Ave, Med Sci S309, University of California, San Francisco, San Francisco, CA 94143-0412 (tom.lietman@ucsf.edu).

(to overcome high variance among villages), to refrain from giving any further treatments over the course of the surveillance, and to include untreated villages (to assess any secular trend). Herein, we investigate whether ocular chlamydial infection returns to the community in the long term after a single mass antibiotic treatment in a high-prevalence setting. Multiple villages in Ethiopia were followed up for 24 months after receipt of a single mass treatment. Untreated villages were randomly chosen and enrolled 12 months into the program to allow estimation of a secular trend. Children aged 1 to 5 years were monitored because this age group is known to have the highest prevalence of ocular chlamydial infection in the community[6,14] and may form a core group for transmission.[8]

METHODS

In March 2003, a simple random sample of 8 peasant associations was chosen from the Enemore/Ener district of the Gurage Zone, Ethiopia. A peasant association is a standardized administrative unit defined by the Ethiopian government and, in this district, typically consists of 5 villages (range, 2-6 villages). One village was randomly selected from each peasant association and was offered mass antibiotic treatment for trachoma. Selecting villages from different peasant associations minimized contact between study villages. The mean distance to the next closest study village was 6.6 km (range, 5.0-10.1 km). A census was conducted to enumerate all village residents. Those aged 1 year or older were offered single-dose oral azithromycin (1 g in adults or 20 mg/kg in children as a directly observed treatment). Pregnant women, children younger than 1 year, and those allergic to macrolides were offered a 6-week course of topical 1% tetracycline ointment (applied twice daily to both eyes, not directly observed). The single mass treatment took place in April 2003.

At baseline (pretreatment) and at 2, 6, 12, 18, and 24 months after treatment, all children aged 1 to 5 years were assessed for the presence of ocular chlamydial infection. This age group was chosen because it is most likely to harbor chlamydia and because children aged 6 years and older attend school, making it more difficult to consistently locate them to perform assessments. Informed consent was obtained orally from the parent or guardian of each child prior to entering the study. In each patient, the right upper eyelid was everted and a Dacron swab passed across the tarsal conjunctiva 3 times, rotating approximately 120° between each pass.

Twelve months after treatment, an additional 2 untreated villages were randomly selected from each of the original 8 peasant associations. One peasant association consisted of only 2 villages, allowing only a single untreated village to be chosen. Children aged 1 to 5 years in these 15 untreated villages were assessed for ocular chlamydial infection in the same manner as in the treated villages. Immediately after monitoring was completed, all village residents in these 15 untreated villages were offered antibiotic treatment as part of the Orbis-Ethiopia trachoma program.

In addition to monitoring all children aged 1 to 5 years at all visits, at 18 months a sample of 25 individuals older than 5 years was randomly selected from the census for villages using the random number generator in Microsoft Excel (Microsoft Inc, Redmond, Wash). This was to facilitate estimating the prevalence of ocular chlamydial infection in the entire community.

Two types of field controls were obtained in a randomly selected 10% of children (not to exceed 5 per village): a duplicate control (a conjunctival swab identical to and taken immediately after the initial study swab) and a negative field control (a swab passed through the air 1 in [2.54 cm] above, but not touching, the patient's conjunctiva). Examiners then changed gloves for the next patient. All samples were kept at 4°C in the field and frozen at –20°C within 6 hours. The swabs were shipped at 4°C to San Francisco, Calif, where they were stored at –70°C until processed. The Amplicor polymerase chain reaction (PCR) test (Roche Diagnostics, Branchburg, NJ) was used to detect chlamydial DNA.

Pretreatment samples were tested individually. Posttreatment samples from the same village were randomized and pooled into groups of 5, with a possible remainder pool of 1 to 4 samples. Each pool was then tested according to the Amplicor protocol. Pooling samples does not work well at the high prevalence found before treatment[15] because essentially all pools will be positive. After treatment, the prevalence of infection is lower, thus allowing for pooling of swabs from each village rather than processing samples individually. In the posttreatment samples with higher prevalence, pooling 5 samples was not productive, so if two thirds or more of the pools were positive, the individual samples were repooled into groups of 2.[15] If any pool produced an equivocal PCR result, all samples from the pool were retested individually.

The prevalence of ocular chlamydial infection in each village was obtained by maximum likelihood estimation.[15] Essentially, the number of positive individual samples most likely to have resulted in the observed pooled results was chosen as the estimate for that village. While this procedure allows for accurate estimation of the prevalence in the village, it does not identify positive individuals without further testing.[15,16] Negative field control swabs from the same village visit were also pooled (typically, 5 samples per pool). If a pool was positive, the individual samples were individually tested. Duplicate controls from the same village visit were pooled and compared with the pool of matched swabs for those same individuals. If the pooled results were discordant, the individual samples were tested.

Analyses were performed at the village level. Correlation of the prevalence in the same village over time was accounted for in a linear regression

Table. Prevalence of Ocular Chlamydial Infection in Children Aged 1 to 5 Years in Treated and Control Villages*

		Prevalence of Infection, % (No. of Children/Total)							
	Treated Village Antibiotic Coverage, %	Treated Village						Untreated Control Villages at 12 mo	
		Baseline	2 mo	6 mo	12 mo	18 mo	24 mo	Control 1	Control 2
Village									
1	96.9	36.0 (9/25)	0 (0/26)	0 (0/23)	0 (0/26)	0 (0/28)	0 (0/49)	0 (0/23)	2.2 (1/45)
2	89.0	58.1 (43/74)	13.9 (11/79)	25.7 (18/70)	19.2 (14/73)	31.0 (22/71)	28.6 (18/63)	2.9 (1/35)	14.0 (14/100)
3	. . .†	31.7 (39/123)	10.1 (10/99)	15.9 (21/132)	20.3 (29/143)	14.3 (9/63)	17.5 (22/126)	22.2 (4/18)	41.5 (27/65)
4	92.0	64.8 (35/54)	11.5 (6/52)	0 (0/74)	6.8 (3/44)	16.3 (8/49)	20.9 (9/43)	6.1 (3/49)	NA‡
5	86.7	38.7 (29/75)	4.3 (3/70)	10.6 (7/66)	4.9 (4/81)	9.6 (7/73)	6.0 (4/67)	29.5 (23/78)	18.5 (5/27)
6	92.4	31.0 (27/87)	1.3 (1/77)	6.0 (3/50)	0 (0/73)	4.1 (3/73)	4.8 (2/42)	0 (0/58)	0 (0/33)
7	92.2	44.9 (22/49)	0 (0/45)	0 (0/39)	2.1 (1/47)	0 (0/43)	5.8 (3/52)	37.0 (34/92)	14.9 (10/67)
8	89.9	42.9 (12/28)	0 (0/26)	0 (0/32)	0 (0/26)	3.2 (1/31)	7.1 (2/28)	30.9 (17/55)	38.3 (23/60)
Mean overall prevalence (95% CI)		43.5 (35.0-52.0)	5.1 (1.1-9.2)	7.3 (0.7-13.9)	6.7 (0.8-12.5)	9.8 (2.5-17.1)	11.3 (4.5-18.1)	17.2 (4.8-29.6)	

Abbreviations: CI, confidence interval; NA, not applicable.
*Polymerase chain reaction participation rates were as follows: baseline, 90.2%; 2 mo, 83.0%; 6 mo, 85.7%; 12 mo, 91.8%; 18 mo, 69.7%; 24 mo, 84.4%; and in control villages at 12 mo, 100%.
†Ellipses indicate missing data because of incomplete records.
‡This peasant association only had 2 villages, so only 1 control village could be chosen.

model using generalized estimating equations (autoregression 1 model; STATA version 7.0, StataCorp, College Station, Tex). Comparisons between villages treated at baseline and control villages within the same peasant association enrolled 12 months later were performed using linear regression, treating peasant association as a random effect (using STATA version 7.0). Ethical approval for this study was obtained from the Committee for Human Research of the University of California, San Francisco, and the Ethiopian Science and Technology Commission; the study was carried out in accordance with the Declaration of Helsinki.

RESULTS

At baseline, the census recorded 571 children aged 1 to 5 years in the 8 study villages. Of these, 515 children (90%) were examined for ocular chlamydial infection at baseline. For the follow-up examinations, the mean PCR participation rate was 83%. Antibiotic treatment covered an average of 91.3% of each community (95% confidence interval [CI], 88.9%-93.7%). All duplicate control swabs were concordant (n=194). However, it should be noted that in a larger study using the same methods, only approximately 95% of duplicate swabs were concordant.[5] A single negative field control (1/194 swabs [0.5%]) was positive (0.9% were positive in the larger study[5]).

Prior to treatment, the mean prevalence of infection in children aged 1 to 5 years by village was 43.5% (95% CI, 35.0%-52.0%) (TABLE). By 2 months after treatment, the mean prevalence of infection had decreased to 5.1% (95% CI, 1.1%-9.2%), which was significantly lower than baseline ($P<.001$). By 24 months after treatment, the mean prevalence of infection had risen to 11.3% (95% CI, 4.5%-18.1%; $P=.001$). Infection returned into the community at an average absolute rate of 3.2% per year (95% CI, 1.3%-5.1% per year; $P=.001$ by autoregression 1 model). The 2 villages with the highest prevalence at baseline (64.8% in village 4 and 58.1% in village 2) also had the highest prevalence at 24 months (20.9% and 28.6%, respectively).

The prevalence of infection varied considerably by village; 7 of the 8 villages had more infection in children at 24 months than at 2 months (Table). In the remaining village, infection in children was reduced from 36% at baseline to 0 at all 5 subsequent visits. This village had the second smallest number of preschool-aged children of the 8 treated villages and had the highest antibiotic coverage (97%). There were also 0 infections in the random sample of the rest of the community in this village at the 18-month visit.

The mean prevalence of infection in all 8 villages at 18 months in a randomly selected 147 individuals older than 5 years was 1.8% (range, 0%-7.1%) compared with 9.8% in 1- to 5-year-olds. The age range of randomly selected individuals was 6 years to 70 years (mean, 27.7 years [SD, 17.7 years]). The sample constituted, on average, approximately 6% of the population of a village. There was no statistically significant difference in the age distribution ($P=.09$ by Kolmogorov-Smirnov test) or sex distribution ($P=.99$ by χ^2 test) between this sampled older population at 18 months and the census population.

Villages enrolled at 12 months that had not been previously treated (n=15) had a mean prevalence of 17.2% (95% CI, 9.5%-24.9%) (Table), significantly lower than that found in villages enrolled 12 months earlier ($P<.001$), suggesting the presence of a secular trend. Twelve months after antibiotic distribution, treated villages had a significantly lower prevalence (6.7%) than villages just being enrolled ($P=.03$), indicating that a treatment

effect was still present at 12 months. There was no statistically significant difference in the age distribution of the sampled children in the treated and control villages at 12 months ($P = .84$ by Kolmogorov-Smirnov test).

COMMENT

The long-term effect of a single mass antibiotic distribution for trachoma has been a subject of debate. Some believe that with high coverage, ocular chlamydial infection can be reduced so effectively that it will never return.[6,10-12] Others think that after just 1 treatment, infection will eventually return and that further treatments or other measures will be necessary to achieve elimination.[3,5,8,17] There is little doubt that infection dramatically declines in the first few months after a single mass treatment.[4-6,18] In 1 study, infection appeared to gradually return between 2 and 6 months after treatment, but communities were then retreated, making longer follow-up impossible.[5] Single villages in Egypt, Tanzania, and Gambia were monitored for approximately 12 months after a single round of mass azithromycin distribution (3 single doses given 1 week apart).[4] Infection increased slightly from 2 to 12 months, although not significantly so, making it difficult to tell whether infection would never return or whether it was doing so very slowly. In a low-prevalence region in Gambia, 14 villages were followed up for 17 months after a single antibiotic distribution.[19] Although infection in the community as a whole had declined, there was a suggestion that infectious loads in children were increasing. Only a single infection was found in a Tanzanian village 24 months after 1 mass azithromycin distribution. However, 3 subsequent biannual distributions of topical tetracycline to clinically active cases may have contributed to this success.[6] Although these previous studies are encouraging, none have monitored enough villages in a hyperendemic area for a sufficient time to determine the long-term effect of a single distribution.

Herein, we addressed this question in a high-prevalence setting, monitoring 8 villages for 24 months after a single mass treatment alone. In 1 village, we were unable to identify any infection at any point after treatment, consistent with local elimination. In each of the other 7 villages, the prevalence of infection in children aged 1 to 5 years was higher at 24 months than at 2 months. In some cases, the return was quite rapid. On average, there was a statistically significant return of infection into the villages. Some have believed that a nonzero threshold may exist below which infection cannot sustain itself and will not return; in population biology, this is sometimes termed an Allee effect.[11] These results do not support such a phenomenon in trachoma control because infection increased even from very low levels.

Although infection was returning, it had not reached baseline levels by 24 posttreatment months in any of the 8 villages. Several reasons for this can be hypothesized. In cases in which treatment does not eliminate infection from an individual, it may still reduce the infectious load; it may then take months for an individual to return to an infectious state, delaying spread back into the community.[6] Twenty-four months may not be long enough for the prevalence to return to the pretreatment level. Also, mass treatment may eliminate some strains of chlamydia from a community; if antigenic diversity is important for chlamydia to partially evade the human immune system, then a less diverse population may never again attain a high prevalence.[10] It is conceivable that treatment eliminates the most sensitive strains of chlamydia, allowing only relatively resistant strains to survive. If this resistance imposes a fitness cost on the organism, then the prevalence of infection may never reach that of baseline. However, it should be noted that resistance in chlamydia is difficult to assess and that no significant macrolide resistance has yet been found.[20]

Perhaps the most likely reason that infection has not reached its pretreatment level in this and other studies is the presence of a secular trend. Ocular chlamydial infection may be gradually declining, even in the absence of a trachoma control program. Many factors could explain a decrease of infection in untreated villages from one year to the next, such as heavy rains, drought, or famine. Such a trend could decrease the observed rate of return. A significant secular trend could easily predominate over a slow return of infection, perhaps explaining the remarkable success of antibiotic programs in hypoendemic areas of Gambia[19] and Nepal.[21] There is evidence that clinically active trachoma may be disappearing in several countries in the absence of a trachoma program.[21-24] In a single Gambian village, the prevalence of active trachoma fell from 66% to 4% over a 28-year period starting in 1959. The only intervention during this time was the distribution of topical tetracycline from 1959 to 1961.[22] In a district in Malawi from 1983 to 1999, the prevalence of active trachoma in children declined from 37% to 14%, with no trachoma control program in place.[24] In an area of western Nepal, clinically active trachoma fell by at least 15% over 6 months, even before antibiotic treatments were administered.[21] In the current study in Ethiopia, we found that villages enrolled at 12 months had significantly less infection than the villages enrolled at baseline, even though those enrolled later had received no antibiotic treatment and no specific nonantibiotic measures other than information delivered in a national radio broadcast. We did not observe any specific environmental changes that may have accounted for the decline. A secular trend may have been present even in this hyperendemic region of Ethiopia.

One limitation of this study is the difficulty of studying communities in isolation. It is possible that treatment of 1 village in a peasant association may have reduced the prevalence in the other villages, including the villages chosen as controls, perhaps through lower intervillage transmission. We did not assess the prevalence of infection in these untreated villages 12 months earlier, as it was considered unethical to offer the communities examinations without treatment. Thus, it is pos-

sible that the prevalence of infection in these villages was lower at baseline, even though they were randomly selected from the same pool as the treated villages. Also, we did not observe any mass migration or holiday traveling that has been proposed as a factor for recurrent disease in other settings.[19]

Another limitation is the difficulty in identifying every infection in a village. While examination coverage of the preschool-aged group most likely to harbor infection was relatively high, we only sampled a small fraction of the rest of the community, and only at the 18-month time point. In addition, even 24 months may not be a long enough period to observe the trend in return of infection. Finally, our examination schedule every 6 months was too crude to assess incidence.

Our results suggest that if infection is not eliminated by a single mass antibiotic treatment, then it predictably returns into the community, at least in this hyperendemic area in 1- to 5-year-old children. However, infection comes back slowly and does not approach baseline prevalence even by 2 years. This return may be dampened to some extent by a secular trend in the area. Regardless, repeated treatments or other measures will be necessary for elimination of infection, as recommended by WHO.[2,3,5,7,8] A single treatment will not suffice.

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Study concept and design: Alemayehu, Melese, Lakew, Yi, Whitcher, Gaynor, Lietman.
Acquisition of data: Chidambaram, Alemayehu, Melese, Lakew, Yi, Cevallos, Zhou, Maxey, Lee, Shapiro, Srinivasan, Whitcher, Gaynor, Lietman.
Analysis and interpretation of data: Chidambaram, House, Zhou, Maxey, Lee, Porco, Lietman.
Drafting of the manuscript: Chidambaram, Porco, Whitcher, Lietman.
Critical revision of the manuscript for important intellectual content: Chidambaram, Alemayehu, Melese, Lakew, Yi, House, Cevallos, Zhou, Maxey, Lee, Shapiro, Srinivasan, Gaynor, Lietman.
Statistical analysis: Chidambaram, Zhou, Maxey, Lee, Porco, Lietman.
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