Ribosomal RNA Evidence of Ocular *Chlamydia trachomatis* Infection Following 3 Annual Mass Azithromycin Distributions in Communities With Highly Prevalent Trachoma

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Twelve trachoma-hyperendemic communities were treated with 3 annual mass azithromycin distributions. Children aged 0–9 years were monitored 1 year following the third treatment. An RNA-based test detected ocular chlamydial infection in more children than did a DNA-based test (6.9% vs 4.2%), and in a larger number of communities (8 vs 7).

Trachoma is a blinding disease caused by ocular infection with *Chlamydia trachomatis*. The World Health Organization (WHO) has targeted trachoma for elimination as a public health concern by the year 2020. Mass antibiotic distributions are an important component of WHO's trachoma elimination strategy. Current guidelines recommend annual mass azithromycin treatments in districts with sufficient trachoma, with reassessment after 3 annual treatments [1].

Most studies that have monitored for trachoma after 3 mass azithromycin distributions have reported only the clinical signs of trachoma. However, there is poor agreement between the clinical signs of trachoma and ocular chlamydial infection after mass antibiotic treatments, likely because clinical signs persist for months after ocular chlamydia has been cleared [2]. The few studies that have monitored ocular chlamydia after 3 repeated mass antibiotic distributions have done so using commercially available nucleic acid amplification tests (NAATs) targeting chlamydial DNA [3, 4]. Recently, studies in both the trachoma and sexually transmitted disease literature have demonstrated that RNA-based NAATs are more sensitive than these DNA-based tests for detecting chlamydia in an individual [5–7]. RNA-based tests have an additional target capture step and a more abundant genetic target, either of which may contribute to the increased sensitivity. It is possible that communities without any DNA evidence of ocular chlamydia could nonetheless have infection detectable by an RNA-based test—although evidence for this was not found in a previous study [8]. In this report, we use both RNA-based and DNA-based tests to monitor 12 trachoma-hyperendemic communities that have been treated with 3 annual mass azithromycin distributions.

METHODS

During a cluster-randomized clinical trial conducted in Ethiopia, 12 randomly selected *subkebeles* (subdistricts) were treated with 3 annual mass azithromycin treatments (clinicaltrials.gov identifier NCT00322972) [12]. We performed an enumerative census and mass azithromycin distribution in all 12 subkebeles at months 0, 12, and 24 of the study. At each distribution, auxiliary health workers offered a single dose of directly observed azithromycin (1 gram for adults, 20 mg/kg for children) to all persons aged ≥1 year.

We performed monitoring in a randomly selected sentinel community from each subkebele. In each of the 12 sentinel communities, 50 randomly chosen children aged 0–9 years underwent conjunctival examination and swabbing at baseline and month 36 (ie, 1 year after the third mass treatment). Separate random samples of children were selected at each time point, based on the most recent census. At each monitoring visit, 1 of 13 trained examiners assessed the upper right tarsal conjunctiva for follicular trachomatous inflammation (TF) and intense trachomatous inflammation (TI) according to the WHO simplified grading system [9]. The examiner then passed a Dacron swab 3 times over the upper right tarsal conjunctiva. At month 36, an additional swab for chlamydial RNA was collected in a similar fashion, using swabs and transport media from the APTIMA-CT Unisex Swab Specimen Collection Kit (Gen-Probe). RNA swabs were always collected after Dacron swabs. Examiners used the

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same pair of gloves for the Dacron and RNA swabs for each study subject, changing into a new pair of gloves for each new subject. We collected negative control swabs on 5 randomly selected children per community for each test by passing a swab within 1 inch of, but not touching, the tarsal conjunctiva.

Dacron swab specimens were stored and transported frozen, and then tested for *C. trachomatis* DNA using the AMPLICOR PCR assay (Roche Diagnostics). Dacron swab specimens from baseline were analyzed as pools of 2 swabs randomly selected from the same community, with maximum likelihood estimation used to determine the number of positive individual tests most likely to have resulted in the pooled results. Dacron swab specimens from month 36 were analyzed as pools of 5 swabs, with individual testing of any positive pools. RNA swab specimens were stored and transported to San Francisco at room temperature; swabs were processed using the APTIMA-CT assay to detect *C. trachomatis* ribosomal RNA, also in pools of 5 with individual testing of positive pools.

We compared community prevalences between baseline and month 36, and between DNA- and RNA-based tests, using a Wilcoxon matched-pair signed-rank test. We estimated community-level and household-level clustering of outcomes by calculating the intraclass correlation coefficient (ICC) on the logit scale, using mixed effects logistic regression with household nested in community as a random effect [10]. We determined statistical significance from likelihood ratio tests comparing the full model to nested models without one of the random effects. Similar models were used to assess for associations with sex and age (<5 years vs ≥5 years). All analyses were performed with Stata software, version 10.0.

RESULTS

We performed baseline examinations on 584 children aged 0–9 years. The mean prevalence of DNA evidence of ocular chlamydia was 41.9% (95% confidence interval [CI], 31.5–52.2; Table 1). Antibiotic coverage in the 3 annual mass azithromycin distributions averaged 80.9% (±13.3%), 92.1% (±5.0%), and 87.3% (±11.8%) at the first, second, and third treatments, respectively.

Follow-up monitoring was performed on 583 children from 370 households at month 36 (1 year after the last azithromycin distribution; Table 1). In total, 25 children from 20 households and 7 communities had DNA evidence of ocular chlamydia (mean prevalence, 4.2% [95% CI, .3%–8.0%]; $P = .002$ compared to baseline), and 41 children from 35 households and 8 communities had RNA evidence (mean prevalence, 6.9% [95% CI, .4%–13.3%]). For both tests, the distribution of infection was highly skewed, with 80% of infected children living in just 3 of the 12 communities. Although the DNA- and RNA-based tests were closely correlated (Spearman $\rho = 0.95$, $P < .001$), the ocular chlamydia prevalence estimate was higher for the RNA-based test compared to the DNA-based test ($P = .02$). The correlation between the community prevalence of ocular chlamydia and the prevalence of clinically active trachoma (TF and/or TI) was similar when using the RNA-based test ($\rho = 0.62$, $P = .03$) or the DNA-based test ($\rho = 0.64$, $P = .02$). All swabs that were positive for DNA were also positive for RNA. No negative control swabs collected at month 36 tested positive for chlamydial DNA (n = 60) or RNA (n = 59).

We found evidence for clustering of chlamydial RNA within communities (ICC = 0.35 [95% CI, .06–.64]; likelihood ratio test $P < .001$) and within households of the same community (ICC = 0.78 [.55–1.00]; $P = .001$); we observed similar results for chlamydial DNA (ICC = 0.38 [.01–.48]; $P < .0001$ and ICC = 0.99 [.97–1.00]; $P < .001$, respectively). Clinically active trachoma was also found to cluster within both communities (ICC = 0.06 [95% CI, .01–.12]; likelihood ratio test $P < .001$) and households (ICC = 0.23 [95% CI, .04–0.42]; $P = .03$). We found no association between the detection of chlamydial RNA and sex ($P = .35$) or age ($P = .88$), nor between chlamydial DNA and sex ($P = .15$) or age ($P = .21$). In contrast, children with clinically active trachoma were more likely to be male (odds ratio [OR], 1.56 [95% CI, 1.05–2.31]) and <5 years of age (OR, 2.82 [95% CI, 1.79–4.46]).

DISCUSSION

After 3 annual mass azithromycin distributions to an area with highly prevalent trachoma, three-quarters of communities had a prevalence of chlamydial RNA of <5%. In one-third of communities, we were unable to detect a single ocular chlamydial infection. However, 3 communities still had a relatively high prevalence of ocular chlamydia after mass antibiotics. This suggests that repeated mass antibiotic distributions may be capable of eliminating ocular chlamydia in some communities with severe trachoma, but pockets of high transmission will likely persist, which may make elimination of the larger area more difficult. This finding is consistent with reports of persistent low levels of ocular chlamydia after ≥3 years of programmatic mass antibiotic distributions, hygiene promotion, and sanitation improvements for trachoma [3, 4].

Of the 8 communities with evidence of chlamydial infection, 6 showed a higher prevalence with the RNA test compared to the DNA test. One community was classified as having eliminated infection by the DNA-based test but not by the RNA-based test. In this discrepant community, the RNA-based test detected only a single case of ocular chlamydia. Thus, although the RNA-based test appears to be a more stringent classifier of elimination, it is unclear whether the increased sensitivity of this test would be meaningful to trachoma programs.

Table 1. Prevalence of Clinically Active Trachoma and Ocular Chlamydial Infection Before Mass Treatments (Month 0), and 1 Year After 3 Annual Mass Azithromycin Treatments (Month 36)

	Before Treatment				Treatment	After 3 Mass Treatments					
	% (No./Total)				Mean (SD)	% (No./Total)					
Community	TF/TI[a]		DNA[b]		% Coverage[c]	TF/TI[a]		DNA[b]		RNA[d]	
1	89.6	(43/48)	44.9	(22/49)	83.4 (9.2)	46.9	(23/49)	18.4	(9/49)	28.6	(14/49)
2	64.0	(32/50)	46.0	(23/50)	73.5 (12.3)	65.3	(32/49)	8.2	(4/49)	10.2	(5/49)
3	59.2	(29/49)	53.1	(26/49)	90.4 (5.2)	50.9	(27/53)	0.0	(0/53)	0.0	(0/53)
4	72.0	(36/50)	52.0	(26/50)	97.8 (2.3)	41.7	(20/48)	0.0	(0/48)	0.0	(0/48)
5	84.0	(42/50)	56.0	(28/50)	92.0 (5.3)	29.4	(15/50)	2.0	(1/50)	4.0	(2/50)
6	40.8	(20/49)	14.0	(7/50)	91.7 (6.6)	38.0	(19/50)	2.0	(1/49)	2.0	(1/49)
7	94.4	(34/36)	16.7	(6/36)	85.6 (3.0)	48.5	(16/33)	0.0	(0/33)	3.0	(1/33)
8	42.0	(21/50)	36.0	(18/50)	94.8 (3.7)	45.8	(22/48)	2.1	(1/48)	4.2	(2/48)
9	92.0	(46/50)	58.0	(29/50)	86.1 (5.0)	63.5	(33/52)	13.5	(7/52)	26.9	(14/52)
10	62.5	(30/48)	22.4	(11/49)	78.4 (30.0)	18.0	(9/50)	0.0	(0/51)	0.0	(0/51)
11	80.0	(40/50)	62.0	(31/50)	85.6 (4.4)	32.6	(14/43)	0.0	(0/45)	0.0	(0/45)
12	44.4	(20/45)	41.2	(21/51)	82.1 (11.5)	40.7	(22/54)	3.7	(2/54)	3.7	(2/54)
Mean (95% CI)	68.7	(56.3–81.2)	41.9	(31.5–52.2)	86.8 (82.4–91.2)	43.5	(35.0–52.0)	4.2	(0.3–8.0)	6.9	(0.4–13.3)

Abbreviations: CI, confidence interval; TF, follicular trachomatous inflammation; TI, intense trachomatous inflammation.

[a] TF and/or TI, according to the World Health Organization simplified grading system [9].

[b] DNA evidence of ocular chlamydia, tested with AMPLICOR-CT.

[c] Mean antibiotic coverage during mass azithromycin distributions at months 0, 12, and 24.

[d] RNA evidence of ocular chlamydia, tested with APTIMA-CT.

According to WHO guidelines, communities that have already received 3 rounds of mass azithromycin should continue receiving mass treatments until the prevalence of TF is <5% [1]. In this study, all communities would have been eligible for continued mass treatment, even though one-third of the communities had no evidence of ocular chlamydia. Continued mass treatments may nonetheless be wise, because ocular chlamydial infection can return rapidly after discontinuation of mass azithromycin distributions in areas with hyperendemic trachoma [11]. Alternatively, programs may wish to minimize the disadvantages of continued mass treatments, such as potential antibiotic resistance and cost, by targeting antibiotic treatments to those most likely to be infected. As this study and others have shown, ocular chlamydia clusters by household, suggesting that strategies to target infected households could be helpful for trachoma elimination.

In conclusion, we showed that 3 annual mass azithromycin treatments may eliminate chlamydial infection in some communities, but not all. Communities with a high prevalence of infection can persist after 3 mass treatments, which may hinder elimination efforts. An RNA-based test detected ocular chlamydia in more communities than a DNA-based test did, although the clinical significance of this is unclear.

Notes

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