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

Jeremy D. Keenan,1,2 Berhan Ayele,7 Teshome Gebre,7 Jeanne Moncada,3 Nicole E. Stoller,1 Zhaoxia Zhou,1 Travis C. Porco,1,2,4 Charles E. McCulloch,6 Bruce D. Gaynor,1 Paul M. Emerson,6 Julius Schachter,1,3 and Thomas M. Lietman1,2,4,5

1F. I. Proctor Foundation, 2Department of Ophthalmology, 3Department of Laboratory Medicine, 4Department of Epidemiology and Biostatistics, and 5Institute for Global Health, University of California, San Francisco; 6The Carter Center, Atlanta, Georgia; and 7The Carter Center, Addis Ababa, Ethiopia

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).

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
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 \textit{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 \textit{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, 3.6–8.0%]; \(P = .002\) compared to baseline), and 41 children from 35 households and 8 communities had RNA evidence (mean prevalence, 6.9% [95% CI, 4.9–13.3%]). 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 (\(P = .062, P = .03\)) or the DNA-based test (\(P = .064, P = .02\)). 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, 0.06–0.64]; likelihood ratio test \(P < .001\)) and within households of the same community (ICC = 0.78 [0.55–1.00]; \(P = .001\)); we observed similar results for chlamydial DNA (ICC = 0.38 [0.11–0.48]; \(P < .0001\) and ICC = 0.99 [0.97–1.00]; \(P < .001\), respectively). Clinically active trachoma was also found to cluster within both communities (ICC = 0.06 [95% CI, 0.01–0.12]; likelihood ratio test \(P < .001\)) and households (ICC = 0.23 [95% CI, 0.04–0.42]; \(P = .03\)). 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.
Thromycin distributions in areas with hyperendemic trachoma infection can return rapidly after discontinuation of mass azithromycin treatments may nonetheless be wise, because ocular chlamydial infection can persist after 3 mass treatments, which may hinder advantages 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, tested with APTIMA-CT, showed, ocular chlamydia clusters by household, suggesting that strategies to target infected households could be helpful for trachoma elimination.

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)

<table>
<thead>
<tr>
<th>Community</th>
<th>Before Treatment</th>
<th>Treatment</th>
<th>After 3 Mass Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TF/TI&lt;sup&gt;a&lt;/sup&gt; DNA&lt;sup&gt;b&lt;/sup&gt; % Coverage&lt;sup&gt;c&lt;/sup&gt;</td>
<td>TF/TI&lt;sup&gt;a&lt;/sup&gt; DNA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>% (No./Total) Mean (SD)</td>
</tr>
<tr>
<td>1</td>
<td>89.6 (43/48)</td>
<td>44.9 (22/49)</td>
<td>82.4 (9.2)</td>
</tr>
<tr>
<td>2</td>
<td>64.0 (32/50)</td>
<td>46.0 (23/50)</td>
<td>73.5 (12.3)</td>
</tr>
<tr>
<td>3</td>
<td>59.2 (23/49)</td>
<td>53.1 (26/49)</td>
<td>90.4 (5.2)</td>
</tr>
<tr>
<td>4</td>
<td>72.0 (36/50)</td>
<td>52.0 (26/50)</td>
<td>97.8 (2.3)</td>
</tr>
<tr>
<td>5</td>
<td>84.0 (42/50)</td>
<td>56.0 (28/50)</td>
<td>92.0 (5.3)</td>
</tr>
<tr>
<td>6</td>
<td>40.8 (20/49)</td>
<td>14.0 (7/50)</td>
<td>91.7 (6.6)</td>
</tr>
<tr>
<td>7</td>
<td>94.4 (34/36)</td>
<td>16.7 (6/36)</td>
<td>85.6 (3.0)</td>
</tr>
<tr>
<td>8</td>
<td>42.0 (21/50)</td>
<td>36.0 (18/50)</td>
<td>94.8 (3.7)</td>
</tr>
<tr>
<td>9</td>
<td>92.0 (46/50)</td>
<td>58.0 (29/50)</td>
<td>86.1 (5.0)</td>
</tr>
<tr>
<td>10</td>
<td>62.5 (30/48)</td>
<td>22.4 (11/49)</td>
<td>78.4 (30.0)</td>
</tr>
<tr>
<td>11</td>
<td>60.0 (40/50)</td>
<td>62.0 (31/50)</td>
<td>85.6 (4.4)</td>
</tr>
<tr>
<td>12</td>
<td>44.4 (20/45)</td>
<td>41.2 (21/51)</td>
<td>82.1 (11.5)</td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>68.7 (56.3–81.2)</td>
<td>41.9 (31.5–52.2)</td>
<td>86.8 (82.4–91.2)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; TF, follicular trachomatous inflammation; TI, intense trachomatous inflammation.
<sup>a</sup> TF and/or TI, according to the World Health Organization simplified grading system [9].
<sup>b</sup> DNA evidence of ocular chlamydia, tested with AMPLICOR-CT.
<sup>c</sup> Mean antibiotic coverage during mass azithromycin distributions at months 0, 12, and 24.
<sup>d</sup> 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**

Acknowledgments. We thank Donald Everett (National Eye Institute [NEI], Bethesda, Maryland), who was the program officer for the underlying clinical trial; the data safety and monitoring committee for the underlying clinical trial, including William Barlow (University of Washington, Seattle, Washington; Chair), Donald Everett (NEI, Bethesda, Maryland), Larry Schwab (International Eye Foundation, Kensington, Maryland), Arthur Reingold (University of California, Berkeley), and Serge Resnikoff (WHO, Geneva, Switzerland); Tadese Alemayehu (the head of the Goncha Woreda health office); Asrat Genet Amnie (the head of the Amhara Regional Health Bureau); the Ethiopian Ministry of Health; and the study personnel who performed the monitoring, including Mitselal Abrahale, Melkam Andualem, Rebecca Beauregard, Manahlosh Berihun, Michael Chen, Temesgen Demile, Tessema Eneyew, Banchu Gedamu, and Melese Temesgen.

Financial support. This work was supported by the National Institutes of Health (NIH), National Eye Institute grants U10 EY016214 and K23EY019071; and National Center for Research Resources, Office of the Director grant number KL2 RR024130, which funds the University of California, San Francisco Clinical and Translational Science Institute), the Bernard Osher Foundation, That Man May See, the Harper Inglis Trust, the Harper Inglis Trust, the Bernard Osher Foundation, That Man May See, the Harper Inglis Trust, the Harper Inglis Trust; the Bodri Foundation, the South Asia Research Fund, Research to Prevent Blindness, and the International Trachoma Initiative.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**


