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Chlamydial infection during trachoma monitoring: are the most difficult-to-reach children more likely to be infected?

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Summary

Objectives—During mass antibiotic distributions for trachoma, certain individuals are difficult to locate, and go untreated. These untreated individuals may serve as a source of community re-infection. The importance of this difficult-to-locate, untreated population is unclear. We sought to determine whether individuals who are difficult to locate were more likely to be infected with ocular chlamydia than those who were easier to locate.

Methods—We monitored 12 Ethiopian communities 1 year after a third annual mass azithromycin treatment for trachoma. Conjunctival swabbing for chlamydial RNA was performed in a random sample of children from each community. If insufficient numbers of children were enrolled on the first monitoring day, we returned on subsequent days.

Results—Of the 12 communities, 10 required more than 1 monitoring day. On average, 16.1% (95% CI 7.9–30.0) of children were enrolled after the initial day. Evidence of chlamydia was found in 7.1% (95% CI 2.7–17.4) of 0–9 year-old children. No ocular swabs collected after the initial day were positive for chlamydial RNA. Children examined after the initial monitoring day were significantly less likely to have ocular chlamydial infection than children seen on the initial day; Mantel-Haenszel common OR = 0 (95% CI 0 – 0.77).

Conclusions—In a setting of repeated annual mass azithromycin treatments, after approximately 80% of individuals have been located in a community, extra efforts to find absent individuals may not yield significantly more cases of ocular chlamydia.

Keywords

sampling bias; chlamydia; RNA; neglected diseases

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INTRODUCTION

Mass azithromycin treatments are highly effective for the ocular strains of chlamydia that cause trachoma. Trachoma treatment programs currently target 80% antibiotic coverage during mass azithromycin distributions (Solomon *et al.* 2006). Low antibiotic coverage may play a role in persistent ocular chlamydial infection (West *et al.* 2007). Conversely, higher antibiotic coverage may make elimination of ocular chlamydia more likely (Melese *et al.* 2004, Ray *et al.* 2007).

The relationship between the costs and benefits of extra efforts to increase antibiotic coverage is unclear. Children who are difficult to locate may have poor access to health services, and therefore may be more likely to be infected with ocular chlamydia. If so, spending additional resources to find and treat these children could be worthwhile. In this study, we address this question by monitoring 12 Ethiopian communities that received three annual mass azithromycin treatments for trachoma, and test whether children who are more difficult to locate have a higher prevalence of chlamydial infection.

METHODS

Study Design

This is a non-pre-specified analysis of a single study time point from a single treatment arm of a National Eye Institute-sponsored cluster-randomized clinical trial for trachoma conducted in Goncha Siso Enese *woreda*, Amhara Region, Ethiopia from 2006–2009. In the clinical trial, 72 *subkebeles* (governmental demographic units) were randomized to one of six trachoma treatment arms (House *et al.* 2009, Porco *et al.* 2009). The current report refers only to one of these treatment arms, in which 12 subkebeles were randomized to annual oral azithromycin treatments over 36 consecutive months (Gebre *et al.*, Submitted, Keenan *et al.*, In press).

Intervention

During each mass antibiotic distribution, all individuals 1 year and older were offered a single dose of directly-observed oral azithromycin (1 g for adults; 20mg/kg for children). Pregnant women and children under 1 year of age were offered topical tetracycline, to be applied twice daily for six weeks.

Outcomes

At the 36-month study time point (one year after the third annual mass azithromycin treatment), clinical examination and ocular swabbing of the right upper tarsal conjunctiva were performed on a random sample of 50 children aged 0–9 years from a sentinel community in each of the 12 subkebeles. The sentinel community was randomly chosen from one of approximately 3 to 5 similar communities in the subkebele. Normally, all 50 children would not present on the first day of data collection, making subsequent monitoring days necessary. We recorded the date that each child received the examination and swabbing. Clinically active trachoma was assessed using the WHO simplified grading system, with clinically active trachoma defined as TF (follicular trachomatous inflammation) and/or TI (intense trachomatous inflammation) (Thylefors *et al.* 1987). Swabs and transport media from the APTIMA-CT Unisex Swab Specimen Collection Kit (Gen-Probe, Inc., San Diego, CA) were used to collect swabs for chlamydial RNA testing, similar to previous studies (Yang *et al.* 2007, Yang *et al.* 2009). A swab was passed firmly 3 times over the right upper tarsal conjunctiva, rotating 120° between each pass. RNA swabs were stored and transported to San Francisco at room temperature. Nucleic acid amplification of

chlamydial 16s rRNA using the APTIMA assay was performed for pools of 5 swabs, with individual tests done on swabs from positive pools.

Statistical analysis

The primary outcome of the study, chlamydial infection, was defined as present if the APTIMA test for chlamydial RNA was positive. Study participants were dichotomized as being present the initial day of data collection, versus being present on a subsequent day. Population-averaged descriptive statistics were performed to take account of the community-clustered survey design. Mixed effects logistic regression models were created to test for differences between the initial and subsequent monitoring days, using monitoring day (initial versus subsequent) as a fixed effect and community as a random effect. For any dichotomous variables with a single common response across all communities on a given monitoring day, 2×2 tables were constructed for each of the 12 communities to depict the presence or absence of the variable in relation to the day of data collection (initial day, versus subsequent days), and a Mantel-Haenszel common odds ratio with exact confidence intervals was calculated using StatXact 3 (Cambridge, MA). All other statistical calculations were performed using Stata 10.0 (College Station, TX).

Ethics

Ethics approval for this study was obtained from the Committee for Human Research of the University of California, San Francisco; Emory University Institutional Review Board; and the Ethiopian Science and Technology Commission. The study was undertaken in accordance with the Declaration of Helsinki.

RESULTS

The median population of 0–9 year-old children in the 12 communities in this study was 95 (IQR 76–118.5). All 12 communities received mass azithromycin treatments at months 0, 12, and 24, and were monitored for trachoma at month 36 (1 year following the most recent treatment). Of 583 monitored children, the mean age was 5.2 years (95%CI 4.9–5.4), and 52.2% (95%CI 46.7–57.7) were male. Ten of 12 communities required subsequent monitoring days to enroll a sufficient number of study participants. Of all children examined, 489 (83.9%, 95%CI 70.0–92.1) were located on the initial examination day, and 94 (16.1%, 95%CI 7.9–30.0) were examined after the initial day. Table 1 shows the characteristics of monitored children stratified by day of examination.

Ocular chlamydial infection was present in 8 communities, and in 41 (7.1%, 95%CI 2.7–17.4) children. The community prevalence of ocular chlamydia was not significantly correlated with the number of children aged 0–9 years in each community (Spearman's =0.46, p=0.13). No ocular swabs collected after the initial monitoring day were positive for chlamydial RNA. Children examined after the initial monitoring day were significantly less likely to have ocular chlamydial infection than children seen during the initial day; Mantel Haenszel common OR = 0 (95%CI 0 to 0.77).

Compared to children seen on the initial monitoring day, those examined subsequently were more likely to be male (OR = 1.85, 95%CI 1.14–3.00). Children seen on subsequent visits were equally likely to have clinically active trachoma as those seen at the initial visit (OR = 1.08, 95%CI 0.65 to 1.80). Changing the definition of clinically active trachoma to (1) TF with or without TI, (2) TI with or without TF, or (3) both TF and TI did not result in a significant association.

DISCUSSION

In this study, conducted 1 year after 3 annual mass azithromycin treatments, children contacted on an initial monitoring day were more likely to be infected with ocular chlamydia than children contacted during subsequent monitoring days. There are several potential explanations for this finding. First, ocular chlamydia is likely transmitted more readily by children who are social and have many community contacts. It is possible that more social children would both have a higher prevalence of ocular chlamydia, and be more likely to present early for examination. Second, although clinically active trachoma is thought to be an asymptomatic disease (Wright *et al.* 2008), it is possible that infected children could have been more symptomatic, and parents may have been more likely to bring them for an eye examination. We found no evidence for this; children examined on the initial day were no more likely to have clinically active trachoma than those seen on subsequent days. Nonetheless, it is still possible that those children with active chlamydial infection had more severe trachomatous disease, which may have led families to seek prompt eye examination. Third, it is possible that unmeasured confounders could account for the association between chlamydial infection and initial monitoring day. Although we found no evidence that community size was a confounder, other unmeasured confounders may have been present. Finally, given the small number of children examined on the subsequent days, chance alone may be responsible for this result.

The results of this study have implications for trachoma programs. Currently, the WHO recommends 80% antibiotic coverage for mass azithromycin treatment programs (Solomon *et al.* 2006). More intensive antibiotic coverage could be worthwhile if the extra efforts were able to treat a sufficient number of individuals with ocular chlamydia. A higher antibiotic coverage could be achieved using various strategies, but one method would be to return to communities on subsequent days. In our study, we performed 84% of examinations on the initial monitoring day, which in the context of a trachoma treatment program would be analogous to an 80% antibiotic coverage target. All infected children identified in these villages were present during the initial monitoring day. This study suggests that after approximately 80% of community members have been located, extraordinary efforts to increase follow-up may not necessarily be of high yield.

The underlying reasons explaining why some children are difficult to locate remain unclear. The children we eventually found after intensive search efforts were more likely to be male, and less likely to be infected with ocular chlamydia. Boys in this area of Ethiopia are often responsible for the household's livestock during the day, and may therefore be far from the village, and less likely to be located. Children not infected with ocular chlamydia could come from healthier families; these families may sense less of a benefit to mass azithromycin, and therefore fail to present during the initial monitoring day. Many other factors could play a role, such as lack of education, inadequate communication, participation fatigue, and pre-existing school, work, or travel commitments (Nakibinge *et al.* 2009, Oyejide and Fagbami 1988, Oliva *et al.* 1997, Rabiu and Abiose 2001, Bowman *et al.* 2002, Wright *et al.* 2010, Shah *et al.* 2010).

In conclusion, this study suggests that if the WHO-recommended 80% antibiotic coverage target can be achieved on an initial day, additional visits to increase antibiotic coverage may not be required, at least in communities that have already been treated with several rounds of mass azithromycin.

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Table 1

Characteristics of children aged <10 years from 12 Ethiopian villages who participated in trachoma monitoring, stratified by the day of monitoring

	Initial Monitoring Day		Subsequent Monitoring Days		
	N	**% (95%CI)**	**N**	**% (95%CI)**	**OR, 95%CI**
Age under 5 years, %	198/489	40.5% (35.0–46.3)	38/94	40.4% (30.2–51.6)	1.00 (0.64–1.56)*
Male gender, %	244/488	50.0% (44.1–55.9)	60/94	63.8% (52.3–74.0)	1.85 (1.14–3.00)*
Active trachoma†, %	215/486	44.2% (36.2–52.6)	37/93	39.8% (28.1–52.8)	1.08 (0.65–1.80)*
Chlamydial RNA+, %	41/487	8.4% (3.3–20.0)	0/94	0% (0–3.8)	0 (0–0.77)‡

*Mixed effects logisitic regression with community as a random effect

†TF (follicular trachomatous inflammation) and/or TI (intense trachomatous inflammation), according to the WHO simplified grading system (Thylefors *et al.* 1987)

‡Mantel Haenszel common odds ratio