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Mass Treatment and the Effect on the Load of *Chlamydia trachomatis* Infection in a Trachoma-Hyperendemic Community

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Abstract

Purpose—Trachoma remains a leading cause of blindness. Determining the most effective antibiotic treatment strategy is essential for the success of country-based trachoma control programs.

Methods—Baseline and 2-month follow-up examinations were performed in a trachoma-hyperendemic village. All residents were offered azithromycin for trachoma after baseline was determined. Infection with *Chlamydia trachomatis* and chlamydial load were determined by PCR. Clinical trachoma status was evaluated. A high chlamydial load was defined as a higher than median chlamydial load among those with infection. Risk factors were examined in multiple logistic regression models. Associations are presented as odds ratios and 95% confidence intervals.

Results—At baseline, 57% of participants were infected with *C. trachomatis*. Although clinical trachoma correlated with infection, 23% of participants with high chlamydial loads showed no clinical signs. Adults represented only 10% of the population with high loads. Treatment significantly decreased the proportion positive in the community and the load in the community. However, 27% of individuals with high loads at baseline who received treatment also were infected at 2 months. Of those, 93% with high loads at 2 months were aged ≤10 years.

Conclusions—Although most of the chlamydial load in this community resided in children, 10% of the high load resided in adults, most of whom did not have follicular trachoma and in whom the infection would be missed under treatment strategies that focus on clinical disease or children. These data support a mass treatment strategy for hyperendemic communities, at least as a first approach. In addition, treatment of children age ≤2 years should be reexamined, as >30% with

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high loads at baseline remained infected at 2 months, despite monitored treatment according to weight.

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Trachoma continues to be the leading infectious cause of blindness worldwide, and the target of a World Health Organization (WHO) initiative for the Global Elimination of Blinding Trachoma by 2020 (GET 2020).[1] Several countries in which trachoma is endemic have started national trachoma-control programs, by using a multifaceted strategy known as SAFE[2]: surgery, antibiotics, face washing, and environmental improvement. The antibiotic, face washing, and environmental improvement components of SAFE are targeted toward reduction of active trachoma within communities.

Where active trachoma is hyperendemic, the prevalence in preschool children may be 70% or higher,[3,4] active trachoma occurs in adults, and high rates of scarring and trichiasis are observed. The greatest load of chlamydial infection also appears to be in children aged <10 years, reaffirming that young children are the primary reservoir of infection for the community.[5] Adults also carry infection in these communities, and although the load may be lower, transmission may still be possible, and the presence of chlamydial antigen may continue to stimulate the scarring process.[6–8]

The purpose of this study was twofold. First, we characterized the load of *Chlamydia trachomatis* in a hyperendemic community by using quantitative PCR and determined the characteristics of those with high chlamydial loads. Second, we evaluated the effect of treatment on chlamydial load within the community, to determine whether single-dose treatment with azithromycin eliminates infection. Such data are critical for determining the most effective treatment strategies in a trachoma-endemic community to decrease transmission.

Methods

This study was performed in the village of Maindi in Kongwa, Tanzania. The longitudinal study has been described in detail elsewhere and is summarized herein for the Kongwa component.[5,6] Research and ethical clearances for all procedures were obtained from the Tanzanian government through the National Institute for Medical Research Institute (NIMR) and through the Johns Hopkins University Institutional Review Board. The study conformed to the tenants of the Declaration of Helsinki.

A baseline census of every household was taken, and a number was applied to the doorway to enable mapping of the geographical location of every house in the village. The census included details on the age, gender, and relationship to head of household of every household member. Household characteristics also were collected, including whether the household was part of a larger compound of contiguous households called a “kaya.”

After the census, a baseline survey was conducted targeting everyone in the village. Trachoma was assessed according to the WHO simplified grading scheme by an experienced trachoma grader who used 2.5× loupes.[9] An ocular specimen was collected for detection of ocular *C. trachomatis* infection and quantification of infection by real-time PCR (Amplicor; Roche Molecular Systems, Branchburg, NJ), as described in detail elsewhere.[5] A sterile

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Dacron swab was held horizontally and passed across the length of the tarsal conjunctiva four times, with the swab rotated a quarter turn with each pass. To ensure consistency in the swab procedures, a single individual was responsible for taking all swabs. Precautions were taken to prevent cross-contamination. Specifically, we ensured that the Dacron tip did not touch anything besides the conjunctiva and the inside of the transport tube, as scissors were not used to cut the swab shaft. The laboratory person did not touch any subject or staff member during the taking of specimens. He also cleaned his hands thoroughly with alcohol between taking specimens. Longitudinal surveys using the same methods were performed on everyone in the village at 2 months.

Each swab was placed dry in a vial, kept cold on frozen freezer blocks in the field for the day, and stored at −20°C until shipped on freezer blocks to the London School of Hygiene and Tropical Medicine for processing. A *C. trachomatis* qualitative PCR assay was used to identify samples positive for the organism (Amplicor; Roche Molecular Systems). Details on processing the samples have been described in a previous paper,[5] but in essence, PCR was performed on eluted samples according to the manufacturer's instructions. Testing for PCR inhibition was performed. If there was evidence of inhibition, samples were retested after diluting an aliquot 1:5 with a 50:50 mixture of lysis buffer (Amplicor CT/NG; Roche Molecular Systems) and specimen diluent. Positive and negative results were then assigned as outlined by the manufacturer. Samples that initially returned equivocal results were designated positive or negative according to the manufacturer's protocol. All positive samples were then processed for quantity of *omp1* (the genomic equivalent of *C. trachomatis*), with a quantitative PCR assay developed and described previously.[5]

Real-time quantitative PCR was performed on a thermocycler (LightCycler; Roche Molecular Systems). The target for quantitative PCR amplification was a 123-bp fragment within constant domain 3 of the *omp1* gene. Each elementary body contains a single copy of this gene. For each sample, quantification was performed twice on two replicate aliquots.[5] Everyone older than 6 months in the village was offered a single dose of oral azithromycin, 20 mg/kg up to a 1-g dose. Children aged ≤2 years were weighed to determine the proper dosage. Children aged <6 months were not treated. Women who stated they were pregnant were offered topical tetracycline if they had no signs of trachoma. Otherwise, they were treated with azithromycin.

Quantification is reported as the number of organisms per swab, as described earlier.[5] Some samples were Amplicor positive, yet LightCycler negative, on one or both runs. These samples were categorized as positive for reasons described elsewhere, and maximum-likelihood estimations were used to impute the number of organisms likely to be contained in the swab.[5] In this article, we refer to loads less than or equal to the median load as "low loads" and loads greater than the median as "high loads." Logistic regression analyses were used to determine the independent contribution of factors to the risk of having an infectious load greater than median value before treatment. General estimating equation (GEE) models with independent correlation structures were used to adjust the confidence intervals for clustering of risk factors at the kaya (household compound) level.

Results

The census tabulated 1020 persons in the village at baseline, of whom 876 (86%) participated in the baseline survey. Individuals who did not participate in the survey were more likely to be male ($P = 0.007$) and aged 11 to 30 years ($P = 0.01$) than participants. Data on infection obtained by the Amplicor test (Roche Molecular Systems) were available for all but three of the participants. Quantitative data were missing on 88 Amplicor-positive specimens at baseline, due to loss of sample at the laboratory. Participants with missing quantitative data were more likely to be aged 16 to 20, 22% versus 11% of those not missing data ($P = 0.015$). Participants missing quantitative data were less likely to be ≤10 years, but there were no differences by gender or household size. Less than 1% of the entire group lived in households with three or fewer persons, and 90% lived in households with six or more persons.

At baseline, 496 (57%) participants were Amplicor positive. Among this group, we examined the distribution of *C. trachomatis* by age and gender (Table 1). The data show the greatest concentration of chlamydial load was in the youngest age groups. Children <7 years carried 90% of the total community load of *C. trachomatis*. Below age 30 years, more of the total community load by age group was in females than in males. Above age 30, infection rates were lower in both sexes, and four males with very high loads (>150 *omp1* units) accounted for a greater total load in males, as >55% of persons >30 years of age had no infection. Individuals with loads above the median value (19.1 *omp1* copies) were seen in all age groups in this community (Fig. 1). Overall, 22% (173/785) of the population had high loads at baseline. Children aged <10 years were more likely to have high loads than were older persons ($P < 0.001$).

The proportion of individuals with high loads at baseline increased with severity of clinical sign (Table 2). Only 8% of individuals with no clinical sign of active trachoma (using the WHO simplified system) had high loads, whereas 40% of those with trachomatous follicles (TF) alone and 51% of those with severe inflammatory trachoma (TI) had high loads.

Of particular interest, 23% (39/173) of those with a high load had no active trachoma. Most of these, 72%, were individuals aged ≥11 years. Moreover, one fourth of those with high loads and no active trachoma lived in households where no one else had trachoma. These analyses suggest no obvious markers of clinical disease or age for finding one quarter of individuals who have high loads of infection and who, therefore, could be sources of transmission.

Young age, female gender, and a high proportion of individuals with high chlamydial loads at baseline living in the same kaya were independent predictors of a high chlamydial load at baseline (Table 3). The higher the proportion of the kaya members that were infected, the greater the likelihood that the participant had a high load at baseline. In fact, individuals living in a kaya where >50% of the other members were infected were 7.2 times more likely to have high loads at baseline than individuals living in a kaya where no other person had a high load (95% confidence interval [CI]: 1.9 –27.3). Tin roofs, presence of flies and presence of a latrine were not predictive of high load at baseline (data not shown).

Treatment coverage of this population was high, with 86% of persons receiving treatment. Among children aged ≤10 years, coverage was 90%. Treatment resulted in a dramatic decline in the chlamydial load (Table 4). However, clearance of infection with no evidence of infection at 2 months declined with increasing load at baseline. Whereas 91% of individuals with a low load at baseline had no infection at 2 months, only 74% of participants with 20+ *omp1* copies had no infection at 2 months after treatment ($P < 0.05$). Of the 33 participants with high load at baseline who also were infected at 2 months, half also had a high load at 2 months. Only one person in the treated population with no or low load at baseline had a high load at 2 months.

The 15 persons who, despite being treated, had high loads again at 2 months are of particular interest, as they represent those individuals who are liable to reinfect others after mass treatment. Among the 162 persons with high loads who were treated, most were persons aged <20 (88%) years, and most (74%) had no infection at 2 months (Table 5). However, all but one of those who had high loads at both time points were aged ≤10 years, compared with 67% of those who were infected at baseline but had no infection at 2 months. In fact, most (9/15) were aged ≤2 years.

Discussion

In this trachoma-hyperendemic community, infection and high chlamydial loads were present in all age and gender groups. High chlamydial loads were most commonly recorded in younger children and in individuals with clinical trachoma. Those with high loads, defined as greater than median chlamydial load, or >19.1 *omp*1 copies, are a critical group, as they are likely to be responsible for transmission within the household and community. They are also a significant fraction of the population, 22%. Our data suggest that although a significant proportion of those with high loads had clinical signs, 23% did not. These individuals tended to be older persons, and some did not reside in households with persons who had clinical signs. Burton et al.[6] found that in hypoendemic villages in The Gambia, most persons with infection did not have clinical signs, although similar to our findings, the prevalence of infection increased with increasing severity of clinical trachoma. In the Gambian population, the prevalence of infection did not vary by age, and only 69% of the chlamydial load was in those aged <10 years. In both our population and in a mesoendemic population in northern Tanzania, 90% of the burden was among children aged <7 years.[5]

It is of interest to determine how many persons with high loads in our population would not be treated by using currently discussed treatment strategies for trachoma control. Theoretically, with mass treatment, all would be targeted and treated, and in fact, in our population, 96% of those with high loads were treated, with overall coverage of the population at 86%. Some programs target only children aged ≤10 years.[11] If only children aged ≤10 years are treated, 69% of those with high load are covered. If treatment were extended to households with children aged ≤10 years, 90% of the entire population would be targeted, because most people live in households with children. Given this, it is more practical to undertake mass treatment.

Treating only persons aged ≤20 years would cover 90% of high loads, and 71% of those with any infection, while targeting 65% of the village population. This approach may seem appealing, as it covers most persons with high loads and may be expected to impact on transmission. However, treatment would not be provided to older adults, who harbor 10% of high loads, or to individuals who have low levels of infection and may still be at risk of ongoing scarring.

Treatment targeted toward individuals with clinical trachoma would miss 23% of those with high loads. In The Gambia, targeting active cases would capture only 25% of individuals with infection and 25% of those with high loads.[6] Others have shown that treatment targeted toward clinically active cases is not cost-effective compared with mass treatment.[10] Although treating persons with active cases and their households misses only 6% of high loads, it still targets 82% of the population and requires screening of every household. Thus, we conclude that mass treatment of the population is still the best approach for hyperendemic communities, provided high coverage is achieved. As we monitored persons for only 2 months for these analyses, we cannot project likely follow-up treatment strategies or frequency.

A novel and significant finding is the large proportion of children aged <2 years with high ocular chlamydial loads, who remained infected 2 months after treatment. Solomon et al.[5] also have found very high loads in children <1 year old in two other trachoma-endemic areas. In the present study, antibiotic treatment was provided by a weight-based approach and directly observed by research staff, so that undertreatment was unlikely to occur.[11] Of children aged ≤2 years, 47% had high loads at baseline. Of the two children in this age group who were not treated, both had high loads at 2 months. Of the children this age who had high loads and were treated, 31% (n = 9) had high loads at 2 months, representing >50% of those who had high loads at both time points, despite treatment. It is unlikely that many of these children were reinfected so quickly, as they either resided in households where everyone was treated or in households where individuals not treated did not have infection. However, we did not treat children aged <6 months for trachoma, nor were they examined. These infants could serve as a source of reinfection, as three of the nine children with high loads at both time points lived in households with infants. They may represent errors in measurement of weight or persons for whom a dose greater than 20 mg/kg is needed for cure. It would be interesting to determine the antimicrobial susceptibility of *C. trachomatis* strains isolated from these children.

There are limitations to our study. The results are based on a large hyperendemic community in Tanzania, but village prevalence can vary.[12] It is reassuring that prevalence of infection and correlation with clinical disease are similar to that previously found in other villages in Kongwa.[4,13] The effect of mass treatment with azithromycin also had effects on infection similar to that observed in previous studies.[14]

There is always the theoretical concern for contamination of specimens during collection in the field. We instituted strict procedures for specimen collection, including having the field laboratory person never touch participants or other team members during collection and the swabs never touch anything other than the eyelid or the interior of the sterile vial. We also

are reassured that contamination did not occur, because evaluation of the order of specimens collected and positivity rates showed that among persons from whom a specimen was collected immediately after a person with high load that was not a family member, only 31% had a high load. This percentage is comparable to persons negative for ocular chlamydia. Thirty-six percent of individuals who were examined after a person (not a family member) without chlamydia had a high load. Although some contamination may always be possible, it is unlikely to explain our findings on heavy loads. Because of the high prevalence of low loads of infection, we cannot rule out contamination of specimens in the field or laboratory as a possible explanation of persons with low loads.

Another limitation was the lack of treatment and examination of infants aged <6 months. The Tanzania National Treatment Control Program did not authorize treatment of infants age <6 months, and such children are difficult to access in Kongwa for several cultural reasons. They may be a source of re-emergent infection, although there were few of them at the time of treatment.

In summary, data on the load of *C. trachomatis* infection in this hyperendemic community confirm that a significant portion of the infectious load resides in young children. However, there are adults, including those with no clinical signs of trachoma, who also carry high loads and could be another source of transmission in this community. Such data support the mass-treatment approach to trachoma control in hyperendemic villages. Although treatment was effective in decreasing the community pool of infection, it appeared to be least effective in very young children with high loads, half of whom still had high loads 2 months later. The causes of this persistence or re-emergence in very young children should be further investigated.

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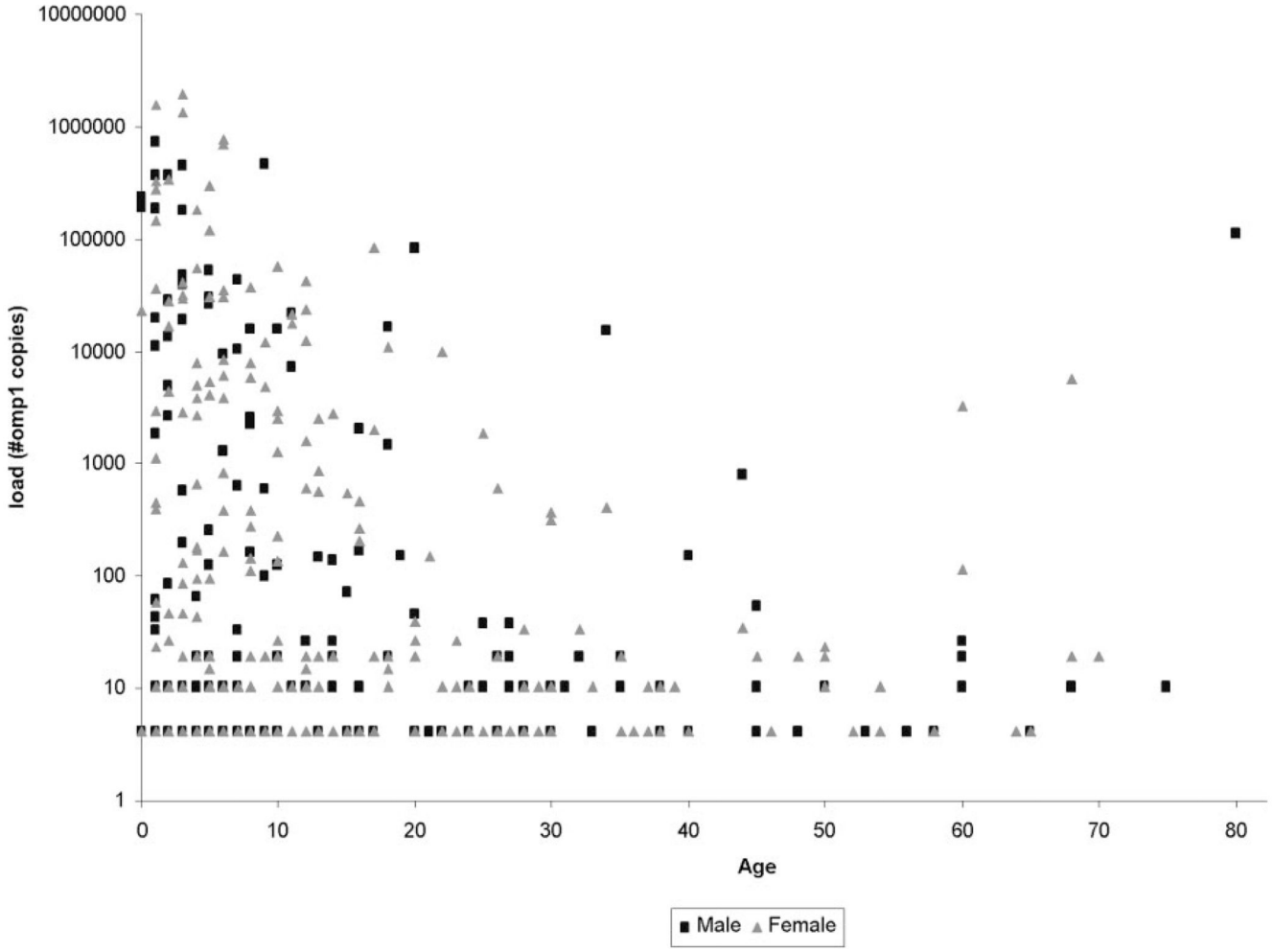

Figure 1. Distribution of chlamydial load in the total population by age and gender.

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Table 1

Baseline Distribution of Chlamydial Load in Those Who Have Infection, and the Percentage with Infection, by Age and Gender, in a Population in Maindi, Tanzania [*]

Age	Males				Females			
	n	Total Load (Copies)[†]	% With Any Infection	% With Active Disease[§]	*n*	Total Load (Copies)[†]	% With Any Infection	% With Active Disease[§]
<5	81	3,062,021	74	81	100	6,936,028	69	78
6–10	67	572,726	51	59	77	1,680,947	62	67
11–20	86	135,896	37	20	95	223,926	56	36
21–30	44	206	39	2	73	13,481	47	13
31–40	27	15,945	37	3	42	543	36	4
41–50	19	898	37	0	23	140	35	4
51+	21	113,882[‡]	52	8	30	9027	37	6
Total	345	3,901,574	50	36	440	8,864,092	54	40

[*] Excluding 88 participants who were positive for infection but are missing quantitative data and three individuals who did not provide a specimen.

[†] Total *omp1* copies per swab.

[‡] One individual accounted for 113,778 copies of this load.

[§] Active disease: TF and/or TI.

Table 2

Number and Percentage of Individuals with No, Low, or High Chlamydial Load at Baseline, According to Clinical Signs of Disease

Clinical Sign	Chlamydial Load			
	None	≤Median Load*	>Median Load	Total
No TF or TI	294 (62%)	143 (30%)	39 (8%)	476
TF only	64 (29%)	66 (30%)	87 (40%)	217
TI ± TF	19 (21%)	26 (28%)	47 (51%)	92
Total	377 (48%)	235 (30%)	173 (22%)	785

*≤19.1 *omp1* copies, includes samples that were Amplicor positive but LightCycler negative (Roche Molecular Systems). Chlamydial load was determined by the number of *omp1* copies per swab. $\chi^2 = 157.4$, $P < 0.0001$.

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Table 3

Predictors of Having a High Chlamydial Load at Baseline

	n	OR	95% CI
Age (y)			
0–5	75/181	9.3	4.8–18.0
6–10	41/144	5.6	3.0–10.5
11–20	34/181	2.8	1.5–5.4
21–30	10/117	1.1	0.6–2.1
31+	13/162	Ref.	—
Gender			
Female	106/440	1.5	1.0–2.1
Male	67/345	Ref.	—
Proportion of remainder of kaya with high loads (%)			
0	25/208	Ref.	—
<25	67/349	1.8	0.8–4.1
25–50	64/187	4.3	1.8–10.0
>50	17/41	7.2	1.9–27.3

Using multivariate logistic regression with generalized estimating equations. OR, odds ratio; CI, confidence interval.

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Table 4

Effect of Mass Treatment on Chlamydial Load at 2 Months after Treatment, According to Baseline Load, among Persons Treated

Baseline Load (*omp1* Copies)	*n*	0 Copies	1–<20 Copies	20+ Copies
0	247	236 (95.6)	10 (4.0)	1 (0.4)
1–<20	169	153 (90.5)	16 (9.5)	0 (0)
20+	126	93 (73.8)	18 (14.3)	15 (11.9)
Total	542	482 (88.9)	44 (8.1)	16 (3.0)

Data are expressed as the number (%) with no, low, and high load at 2 months. Fisher exact test, $P < 0.0001$.

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Table 5

Age Distribution of Participants with High Chlamydial Load at Baseline and No Infection after Treatment, compared with the Age Distribution of Participants with High Loads at both Time Points, among Persons Treated

Age Group (y) of Individuals with High Baseline Load	No Infection after Treatment	High Load of Infection after Treatment
≤5	38 (41%)	13 (87%)
6–10	24 (26%)	1 (7%)
11+	31 (33%)	1 (7%)

Fisher exact test, $P = 0.004$.