Spatial Clustering of Ocular Chlamydial Infection over Time following Treatment, among Households in a Village in Tanzania

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PURPOSE. To observe the spatial distribution of households with high loads of ocular chlamydia in children, before and after mass treatment with azithromycin to determine whether there exists spatial clustering of households with high loads of infection and the spatial scale of the clustering.

METHODS. All residents of a village in Tanzania were invited to participate in the study. A global positioning system unit recorded the location of each house. Mass treatment with azithromycin was offered, with participation above 80%. Active trachoma and swab samples of the conjunctiva were assessed at baseline and at 2, 6, 12, and 18 months after treatment. A k-function analysis was performed to detect clustering of households with high loads of ocular chlamydia in children younger than 8 years.

RESULTS. A total of 1055 villagers were examined during the study; of these, 374 (35.4%) were children younger than 8 years. The total number of households was 215, with 182 (84.6%) households having at least one child. K-function analysis showed clustering of households with high loads of ocular chlamydia at distances up to 2 kilometers (km) at baseline; at 6 months, slight clustering existed within 0.5 km. At 12 and 18 months, high load households clustered at distances up to 1.5 km.

CONCLUSIONS. This analysis suggests that infection spreads between households with children or that nearby households share the same risk factors for infection. Mass treatment has value in lowering infection prevalence within the community, and clustering of households with infection takes up to 1 year to reemerge at the same level as baseline. Retreatment at yearly intervals may interrupt spread of infection. (Invest Ophthal Vis Sci. 2006;47:99–104) DOI:10.1167/iovs.05-0326

Trachoma is a leading infectious cause of blindness worldwide and is hyperendemic in many areas of Africa. Trachoma is caused by an ocular infection with O. lymphatica, of which the clinical manifestation is chronic conjunctivitis, possibly leading to irreversible damage to the cornea and visual loss. Man is the only known host, and infection is passed to susceptible tissues through mechanical means, such as hands or flies. Within communities, the pool of C. trachomatis resides mainly in children.1,2

Previous studies have found that, within households, those living with someone with trachoma are more likely to have trachoma.3,4,5 but there are scant data on whether infection is likely to be passed between children in different households. The purpose of this analysis was to observe the spatial distribution of households with high levels of infection in children previous to and after mass treatment, to determine whether there exists spatial clustering of these households, and, if so, the spatial scale of the clustering.

METHODS

Participants

The village of Maindi in Kongwa, Tanzania, was selected for study based on a previous pilot survey in the area that indicated that trachoma was highly endemic in this community, and funding requirements for a village of approximately 1000 people. All residents of Maindi were eligible to participate in the study.

Census

A baseline census was taken of every house, and all persons living in the house were added to the census listing. The village consisted of 215 households, and subsistence farming is the usual occupation. Households were not tightly packed together nor widely spaced apart. The census included information on age and gender of every household member. A household number was affixed to each doorway to enable geographical mapping of the household and following household members over time. A handheld global positioning system (GPS) unit (Garmin GPS-75; Garmin, Olathe, KS) was taken to the doorway of every household, and three readings were taken of the latitude and longitude position. The average of these readings was used for the coordinates of the household. Consistency of readings was done daily, using a landmark for calibration. The village takes up an area of approximately 6 by 6 kilometers (km), with no immediately neighboring village. The average latitude and longitude reading for Maindi was 6°12’48.2” and 36°46’35.2”, respectively. The median distance to the closest neighboring household was 0.013 km (interquartile range [IQR] = [0.009, 0.028]).

Clinical Examination

Consenting individuals were surveyed and came to a central clinic site to have both eyes examined for signs of trachoma, according to the World Health Organization (WHO) simplified grading scheme.6 where active trachoma refers to WHO grades Trachoma Follicular (TF) and/or Trachoma Infectious (TI). At the clinic, a swab was rubbed across the tarsal plate of the right eye, following a standardized protocol. Each swab was placed in a dry vial and kept cold. In this study, great care was taken to avoid contamination between swabs, where the technician collecting swabs did not touch patients, and the technician flipping lids washed his/her hands between patients.

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Single mass treatment of azithromycin was given following these procedures: 1 g for adults, taken in a directly observed, single oral dose; and for children, 20 mg/kg of weight. Topical tetracycline was offered to women self-reporting as pregnant and with clinical signs of trachoma. Clinical examination and swab samples were taken again at 2, 6, 12, and 18 months after the baseline examination.

Swabs were analyzed with a qualitative PCR assay (AmpliCycler; Roche Molecular Systems, Branchburg, NJ), testing for the presence or the absence of C. trachomatis. In swabs that tested positive, an additional quantitative test (LightCycler; Roche Molecular Systems) was used to determine the number of copies of the omp1 gene on the swab: each C. trachomatis organism (also referred to as “ocular chlamydia”) contains one copy of this gene. However, the quantitative test was not standardized to the amount of material obtained in a swab, so some of the variance in the detected load may be due to variation in the swabbing technique. The analysis of swab samples has been described in more detail elsewhere.2 Participants who tested positive for the presence of C. trachomatis are described in this article as ‘laboratory-positive,’ rather than ‘infected,’ because a positive Amplicor test does not necessarily mean that the person had an infection.

The Johns Hopkins Institutional Review Board and the Tanzania National Medical Research Institute approved all procedures. Informed consent was obtained in accordance with the tenets of the Declaration of Helsinki.

Statistical Analysis

All consenting children younger than 8 years with one or more clinic examinations and who resided in mapped households were included in this analysis. Ocular chlamydia load in the community was highest in this age group,1,2 and spread across households, if it occurs, is likely through children. We were particularly interested in the problem of households with children having high loads of ocular chlamydia and whether these households spread infection to other households over time.

We chose to observe clustering of households with heavy loads of ocular chlamydia because previous work has shown that spreading of infection within a household may require a heavy load of infectious agent, rather than small amounts of the infectious body.3 This analysis focused on probable infection that could spread and lead to spatial clustering over time.

Analyses were restricted to households with at least one child under age 8 years. Households were assigned to ‘high load’ (HL) and ‘no or low load’ (LL) households according to mean ocular chlamydia load in children in the household. HL households had high mean loading of ocular chlamydia, defined as being above the median for mean household loading at baseline, among households with children with Amplicor-positive tests. Households without children or that contained children with Amplicor-negative tests were not used to calculate the median household load. LL households had below the median load or zero mean household loading. Mean household loading was calculated by using an adjusted geometric mean of ocular chlamydia load among children in the household. We adjusted the usual formula so that zeros might be included.

This definition of mean household loading reflects both the numbers of children with laboratory-positive tests and the load of ocular chlamydia within the household, because both are liable to be related to transmission. This analysis focused on detecting clustering and the spatial scale of clustering of high load households in the region by using the statistic $\hat{D}(d)$, as part of a $k$-function analysis.4–9 for each data collection period. Briefly, the $k$-function is a tool for point cluster detection and is defined as the expected number of events a distance $d$ from an arbitrary event (equation 1).

$$\hat{k}(d) = E[\text{number of events} < d \text{ from an arbitrary event}] / \lambda$$

In Equation 1, $E$ refers to the edge-corrected expected value, and $\lambda$ is the density of events in the region of interest, enabling us to compare $k$-functions between different groups regardless of event prevalence.

We calculated the $k$-function by using the Splancs package in R.5 The $k$-function is effective for estimating general tendencies toward clustering for distances that are small compared with the region size, distances that are approximately about half the region’s width or length. Thus, we were only able to estimate clustering for distances approximately 3 km or less.

When the goal is to determine whether households with heavy loading of ocular chlamydia cluster more heavily than those with low or no loads, the focus is on detecting clustering above the normal clustering of households in the region. Taking the difference of the $k$-functions for HL and LL households (equation 2) determines if there is higher clustering in one of the two groups: higher clustering in HL households is seen when $\hat{D}(d)$ is positive. To determine significance of the clustering, bootstrap sampling of the randomization distribution of $\hat{D}(d)$ was done to determine its null distribution, where the null hypothesis is that HL households do not cluster more tightly than LL households. Values of $\hat{D}(d)$ that lie outside the 2.5th and 97.5th percentiles (95% interval) of the null distribution indicate significant difference in clustering for distances $d$. The distance, $d$, gives an idea of the size of the spatial scale of clustering.

$$\hat{D}(d) = \hat{k}_{\text{HL}}(d) - \hat{k}_{\text{LL}}(d)$$

Results

Among mapped households, a total of 1055 Maindi villagers participated in at least one examination during this study: 865 (865/1055, 82%) participated at baseline, with an additional 190 (190/1055, 18%) villagers participating in at least one follow-up examination. Of those participating in at least one follow-up, but not the baseline examination, 117 (117/190, 61.6%) were part of the baseline census; 73 (73/190, 38.4%) joined the study after baseline. There were 851 (851/1055, 80.7%) who received treatment at baseline.

There was a total of 374 (374/1055, 35.4%) villagers who were children younger than 8 years: 269 (269/374, 71.9%) participated at baseline, with an additional 105 children (105/374, 28.1%) coming to at least one of the follow-up examinations. There were 260 children (260/269, 96.7%) who participated in baseline and received treatment; however, among those participating after baseline, only 14 (14/105, 13.3%) had received treatment. The median load of infection in children with laboratory-positive tests was 19.1 copies/swab at baseline. The proportion of children with laboratory-positive tests and high ocular chlamydia loads (loads higher than the median) dropped after baseline treatment, but rates of active trachoma dropped less radically (Table 1). The proportion of laboratory-positive children dropped 46.9% ($P < 0.0001$) between baseline and 2 months, remained approximately the same between 2 and 6 months ($P = 0.25$), dropped again between 6 and 12 months ($P = 0.0001$), and rose a little between 12 and 18 months ($P = 0.052$). The 6- and 18-month time periods corresponded with the rainy season, so this may account for some of the variability in ocular chlamydia prevalence. The rates of active trachoma dropped 15.2% ($P = 0.0003$) between baseline and 2 months but remained approximately the same thereafter.

The total number of households was 215, with 175 (175/215, 81.4%) households having at least one child; among these households, the average number of children was 2.2, with a range of 1–5. Of the households with children, 127 (127/175, 72.6%) had at least one child with a laboratory-positive test at some point in time. There were 87 (87/175, 49.7%) households with 100% of children having treatment; 147 (147/175, 84.0%) households with at least 50% of the children having treatment,
and 24 (24/175, 13.7%) had no children treated. The proportion of households with laboratory-positive children dropped after baseline treatment, and the proportion of households with children having active trachoma dropped as well (Table 2). Among households with laboratory-positive children, mean household loading of ocular chlamydia at baseline had a median of 32.4 copies/swab and IQR of [5.7, 1336.3] copies/swab. At the 2-month follow-up, the median average household load was only 10.1 copies/swab with IQR, [2.4, 215.2], among households with laboratory-positive children.

Figures 1a-e show the relative locations of all 175 households in Maindi that had children living in them. Households considered to have high ocular chlamydia loads are shown in red. At baseline (Fig. 1a), 43% of households were considered to have high loads. This was reduced after treatment with azithromycin to 13% at 2 months after treatment (Fig. 1b), 20% at 6 months (Fig. 1c), 9.5% at 12 months (Fig. 1d), and 24.3% at 18 months (Fig. 1e). As can be seen at the baseline and follow-up maps, much of the ocular chlamydia was reduced in children using mass treatment. HL households (i.e., average household loading was 32.4 copies/swab or higher) remained in the following months, but overall rates of households with laboratory-positive children remained lower than baseline rates (Table 2).

Clustering of HL households occurred at distances \&lt; 2 km, at baseline (Fig. 2a). Two months after treatment, there was no difference in clustering of HL or LL households (Fig. 2b); and at 6 months, slight clustering existed within 0.5 km (Fig. 2c). At 12 and 18 months, there was clustering of HL households, for distances \&lt; 1.3 km (Figures 2d-e). Although laboratory-positive test rates and ocular chlamydia loading did not return to baseline levels by 18 months, HL households did cluster together at distances close to what was found at baseline, suggesting that households with high loading spread ocular chlamydia to nearby homes by 18 months.

Other household outcomes were examined for spatial clustering: households with at least one child with a laboratory-positive test and households with any active trachoma in children. Among households with at least one child with a laboratory-positive test, there was significant clustering at baseline, similar to the difference in \(k\)-functions shown in Figure 2. However, at 6 and 12 months, the clustering among households with at least one child with a laboratory-positive test was borderline significant at distances up to 1 km, and, at 18 months, showed similar clustering to HL households. No clustering effects were seen for households with active trachoma. Households with larger numbers of children might have increased risk of recurrent infection; however, we found no clustering of households with larger numbers of children (1 or 2 children versus 3 or more).

## DISCUSSION

In this Tanzanian village population, we were able to detect clustering of households with high loading of ocular chlamydia among children, at distances up to 2 km. After treatment, clustering of households with high loads in children approached distances slightly less than 2 km at 12 and 18 months after treatment. This finding is consistent with children having a major role in the transmission of ocular chlamydia infection, because they do not usually travel far but do interact with children from neighboring households. The clustering of households with high loads of ocular chlamydia and the increasing cluster size suggests that reinfection after treatment will radiate out from foci of infection that were not treated. Rates of laboratory-positive tests among children dropped after

### Table 1. Clinical Findings and Laboratory Results for Participating Children Aged 0–7 Years in Mapped Households

<table>
<thead>
<tr>
<th>Time</th>
<th>N</th>
<th>% Laboratory Positive</th>
<th>% High Ocular Chlamydia Load*</th>
<th>% Active Trachoma†</th>
<th>% Treated</th>
<th>Missing</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>268</td>
<td>68.3</td>
<td>39.3</td>
<td>76.6</td>
<td>96.7</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>2 Mo</td>
<td>243</td>
<td>21.4</td>
<td>10.3</td>
<td>61.4</td>
<td>—</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>6 Mo</td>
<td>264</td>
<td>26.1</td>
<td>16.7</td>
<td>53.2</td>
<td>—</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>12 Mo</td>
<td>287</td>
<td>12.8</td>
<td>11.2</td>
<td>46.8</td>
<td>—</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>18 Mo</td>
<td>292</td>
<td>19.2</td>
<td>15.1</td>
<td>57.2</td>
<td>—</td>
<td>82</td>
<td></td>
</tr>
</tbody>
</table>

* High loading was defined as the median load of laboratory positive children at baseline or higher (≥19.1 copies/swab).

† Active trachoma refers to WHO grades TF and/or TI.

### Table 2. Clinical and Laboratory Findings by Household

<table>
<thead>
<tr>
<th>Time</th>
<th>N</th>
<th>% HH with at Least One Laboratory Positive Child</th>
<th>% HH with at Least One Child with High Mean Load of Ocular Chlamydia*</th>
<th>% HH with at Least One Child with Active Trachoma</th>
<th>% HH with at Least 50% Children Treated</th>
<th>Missing HH Information N†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>148</td>
<td>75.7</td>
<td>43.5</td>
<td>85.2</td>
<td>84.0</td>
<td>27</td>
</tr>
<tr>
<td>2 Mo</td>
<td>138</td>
<td>29.0</td>
<td>13.0</td>
<td>66.9</td>
<td>—</td>
<td>37</td>
</tr>
<tr>
<td>6 Mo</td>
<td>146</td>
<td>35.6</td>
<td>20.0</td>
<td>63.7</td>
<td>—</td>
<td>29</td>
</tr>
<tr>
<td>12 Mo</td>
<td>147</td>
<td>17.0</td>
<td>9.5</td>
<td>57.0</td>
<td>—</td>
<td>28</td>
</tr>
<tr>
<td>18 Mo</td>
<td>148</td>
<td>24.3</td>
<td>14.2</td>
<td>67.6</td>
<td>—</td>
<td>27</td>
</tr>
</tbody>
</table>

* High mean load was defined as the median adjusted geometric mean of the household loading in children at baseline among households with laboratory positive children or higher (≥32.4 copies/swab).

† All children in household were missing information.
baseline treatment and did not return to baseline rates. Rates of active trachoma did not drop as dramatically. This apparent lack of treatment effect on active trachoma may be due to a lag in time between development and resolution of active signs and the period of laboratory positivity, or to conjunctival changes due to other microorganisms, the manifestation of which appears similar to active trachoma.

A study of clustering of households with trachoma was done in Kahe Mpya, a subvillage in Rombo, Tanzania. Kahye is a small village on the wooded slopes of Mount Kilimanjaro with houses (relatively) close together compared with Maindi, which is an open scrub savannah with houses relatively far apart. This study detected clusters of households with high prevalence of active trachoma with small radial distances (approximately 0.3 km) and low autocorrelation between households at baseline (no longitudinal data were presented). This study used the Kulldorf spatial scan statistic to detect clustering.

Our analysis of clustering of households with active trachoma by using the statistic $\hat{D}(d)$, showed no clustering of active trachoma for any distance in endemic conditions (baseline). The difference between clustering of active trachoma in these two villages may be due to differences in prevalence of disease, although the different statistical methods used to detect clustering might also be a factor. Infection rates and active trachoma prevalence in endemic conditions is much higher, for all age groups, in Maindi. It may be that poor environmental conditions and higher infection levels promote transmission of chlamydia more readily in Maindi, thus leading to virtually constant follicular disease in children.

Our analysis did not examine direct transmission, and it is possible that households with heavy loads of ocular chlamydia simply share the same risk factors for infection. Risk factors (e.g., latrines, proximity to water, and annual seasonal variations) might affect clustering of infected households but were not explored in this analysis. Contamination of swabs, if present, should not have appeared as clustering, because families came to a central clinic site; neighboring households may have come into the clinic at the same time, but this was not controlled.

Although we cannot exclude the possibility of the spread of infection from older children or adults, we deliberately chose young children as the reservoir of infection, because previous work in this population has shown that 90% of those with high ocular chlamydia loads at baseline were children 10 years or younger. Residual infection after treatment was also concentrated in children, with all but two of those with high loads after treatment being children. Spread from older children and adults may well occur, but the locus of infection after treatment is likely to be children. In this area of Tanzania,
imported infection was not a risk factor for re-emergent infection. We argue that the real issue for communities is the degree of coverage and factors that may cause re-emergence within these communities. This analysis demonstrates that if there is residual infection, then there is evidence for clustering and spread over time.

In summary, clustering of households with heavy loading of ocular chlamydia in children occurred at distances up to 2 km in endemic conditions and clustered at distances a little <2 km by 1 year after mass treatment of the village, although prevalence of laboratory positive tests did not return to baseline levels. Our analysis suggests that ocular chlamydia spreads between households with children or that nearby households share the same risk factors for infection, further confirming the value of mass treatment in highly endemic villages, at least at yearly intervals, to interrupt spread of infection.

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