Clinical and Epidemiologic Research

Geospatial Distribution and Clustering of *Chlamydia trachomatis* in Communities Undergoing Mass Azithromycin Treatment

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PURPOSE. We detected spatial clustering of households with *Chlamydia trachomatis* infection (CI) and active trachoma (AT) in villages undergoing mass treatment with azithromycin (MDA) over time.

METHODS. We obtained global positioning system (GPS) coordinates for all households in four villages in Kongwa District, Tanzania. Every 6 months for a period of 42 months, our team examined all children under 10 for AT, and tested for CI with ocular swabbing and Amplicor. Villages underwent four rounds of annual MDA. We classified households as having ≥ 1 child with CI (or AT) or having 0 children with CI (or AT). We calculated the difference in the K function between households with and without CI or AT to detect clustering at each time point.

RESULTS. Between 918 and 991 households were included over the 42 months of this analysis. At baseline, 306 households (32.59%) had ≥ 1 child with CI, which declined to 73 households (7.50%) at 42 months. We observed borderline clustering of households with CI at 12 months after one round of MDA and statistically significant clustering with growing cluster sizes between 18 and 24 months after two rounds of MDA. Clusters diminished in size at 30 months after 3 rounds of MDA. Active trachoma did not cluster at any time point.

CONCLUSIONS. This study demonstrates that CI clusters after multiple rounds of MDA. Clusters of infection may increase in size if the annual antibiotic pressure is removed. The absence of growth after the three rounds suggests the start of control of transmission.

Keywords: trachoma, *Chlamydia trachomatis*, azithromycin

Trachoma is the leading cause of infectious blindness in the developing world.[1] It is the result of repeated infection with *Chlamydia trachomatis*, which results in inflammation, scarring of the conjunctiva, entropion, and trichiasis, with the blinding sequelae of corneal opacification.[2] Trachoma disproportionately affects the poorest parts of the world, and contributes to an ongoing cycle of disability and economic deprivation.[3]

The World Health Organization (WHO) has recommended a four-pronged approach to trachoma elimination; that is, SAFE (Surgery to repair inturned eyelashes, mass administration of Antibiotics [MDA] to reduce the pool of *C. trachomatis*, Face washing to reduce transmission from mucosal secretions, and Environmental improvements to interrupt transmission and prevent re-emergence of infection). The antibiotics portion of these guidelines is an area of ongoing research. In districts or subdistricts where active trachoma prevalence is greater than 10% in children aged 1 to 9 years, the WHO recommends annual community-wide MDA for at least three to five years before impact surveys, with coverage of at least 80% of the

population.[4] This recommendation was based on expert opinion as the results were inconsistent regarding the optimum number of rounds of MDA required to achieve the goal of reducing follicular trachoma (TF) to less than 5%. Some studies have shown as little as one round of MDA can eliminate trachoma in lower prevalence settings.[5–7] However, in communities in Tanzania with initial trachoma prevalence of approximately 12%, at least three rounds of treatment were going to be necessary to reduce infection.[8] Even more may be required for those starting at high prevalence.[9]

It is unclear why some communities require more rounds of MDA to eliminate trachoma. One hypothesis is that *C. trachomatis* infection tends to cluster, and if a cluster is missed by MDA, infection may persist. Several studies have looked at trachoma clustering in communities with differing baseline prevalence. In a longitudinal study of a village with 76.6% baseline prevalence in children ages 0 to 7, clustering of infection was present at baseline, but disappeared after a single round of MDA. However, 12 months after treatment clustering of infection re-emerged and was associated with rebound in

infection prevalence.[10] A separate cross-sectional study[11] in a village with 42% trachoma prevalence in children aged 1 to 9 demonstrated that active trachoma tended to cluster without any treatment.

However, to our knowledge no study has analyzed the presence of clustering during and after three annual rounds of mass drug administration. The presence of clustering may explain the rebound of infection seen in these low prevalence settings after administration of 1 to 2 doses of MDA.[8] If significant clustering is detected in such settings, MDA could be targeted to clustered areas so that infection may be eliminated more quickly and efficiently.

The main objective of this study is to detect geospatial clustering of households with *C. trachomatis* infection and active trachoma in Tanzanian villages receiving MDA over time. Clustering was assessed before and after each round of MDA. We hypothesized that significant clustering persists after MDA.

METHODS

Participants

We randomly selected four villages from a group of 32 villages undergoing active treatment for trachoma through the Partnership for the Rapid Elimination of Trachoma (PRET) Trial. All children under 10 years of age in the four villages selected for this study were examined for trachoma at each data collection period, while in the other 28 villages a random selection of children was examined at each time point. Therefore, these 28 villages did not provide longitudinal data on the children. All villages were located in the Kongwa District, Dodoma Region of Tanzania. The study was approved by the National Institute for Medical Research in Tanzania and the Johns Hopkins University Institutional Review Board. Written informed consent was obtained from guardians for all children after explanation of the nature and possible consequences of the study. The research followed the tenets of the Declaration of Helsinki.

Clinical Exam

All children in the village under 10 years of age were invited to a clinical exam at a central site in their villages at baseline, and 6, 12, 18, 24, 30, 36, and 42 months. Because ocular chlamydia prevalence is highest in children under 10 years, we included only this age range in our analysis. Spread among households is likely through children.[10]

Clinical signs of trachoma were assessed by trained graders using the WHO simplified grading scheme.[12] Active trachoma was classified as either TF or intense inflammatory trachoma (TI). To detect *C. trachomatis* infection, conjunctival swabs were obtained from each child's right eye. The swabs were placed in a tube, kept cold in the field and in Kongwa, and sent within 30 days of collection to The Johns Hopkins International Chlamydia Laboratory for analysis of presence of *C. trachomatis* DNA using Amplicor (Hoffman-La Roche, Indianapolis, IN, USA). Positive and negative controls were included in every sample run. Equivocal samples were rerun to determine positivity.

Treatment

The four villages underwent mass treatment following the surveys at baseline, and 12, 24, and 36 months. Azithromycin was offered to all residents of the community at 20 mg/kg up to 1 g in a single dose. Treatment was observed directly. Children under 6 months of age were offered topical tetracycline, twice per day for 4 to 6 weeks, and treatment

was not directly observed. For each MDA, the antibiotics were distributed by two to six trained Community Treatment Assistants in each community over a period of several days. On the first day antibiotics were distributed from a centralized location, followed by household visits on the subsequent day. If antibiotic coverage was below 80% in children under 10 years after 2 to 3 days, then drug distribution was prolonged until the 80% coverage mark was reached or all children were accounted for and reasons for nontreatment recorded.

Spatial Mapping of Households

An updated census of every household and persons living in the households was completed before each mass drug administration. At baseline, each household in the village was visited and a unique household number was assigned. A Galaxy Tab 2.0 7-inch Android device (Samsung, Seoul, South Korea) was used at the doorway to obtain three readings of longitude and three readings of latitude. The mean value of latitude and longitude obtained from these readings was assigned to the household.

Statistical Analysis

We mapped the geographic distribution of households with children in the appropriate age range for each village (Fig. 1). We classified households as having at least one child with infection (or trachoma, if trachoma was the outcome) or having no children with infection (or trachoma). Using this binary classification, we mapped the spatial distribution of houses with and without children with infection (or trachoma) for all 4 villages over time (Figures available upon request).

We detected clustering of households using the $\hat{D}(d)$ value obtained from a K function analysis.[13] To obtain maximum power, we took all four villages into account when calculating $\hat{D}(d)$. The K function analysis is designed for detection of point clusters. It is defined as the expected number of events a distance d from an arbitrary event and is given in Equation 1 below:

$$\hat{K}(d) = E(number\ of\ events < d\ from\ an\ arbitray\ event)/\lambda$$
$$(1)$$

Here E is the expected value and λ is the density of events in the region of interest. The K function values were calculated using the Splancs package in R.[14]

To determine whether households with infected children tend to cluster more heavily, we took the difference in the K function ($\hat{D}(d)$) between households with infection or trachoma and households without infection or trachoma (Equation 2). If clustering is observed between the households with infected children, $\hat{D}(d)$ will be positive.

$$\hat{D}(d) = \hat{K}_{infected}(d) - \hat{K}_{noninfected}(d) \qquad (2)$$

We detected statistical significance of clustering by using bootstrap sampling of the randomization $\hat{D}(d)$ to obtain its null distribution (2000 iterations). The null hypothesis in this case is that households with infection do not cluster more heavily than households without infection at each time point. We considered values outside of the 95% confidence interval of the null distribution to indicate significant clustering. The distance (d) at which clustering occurs provides a sense of the size of individual clusters of infection. The maximum value of d used for this analysis was half the diameter of the individual villages, which we estimated to be approximately 1 km based on our maps. Plots of K functions (Figs. 2, 3) were magnified for easier viewing with d between 0.00 and 0.30 km shown. There was
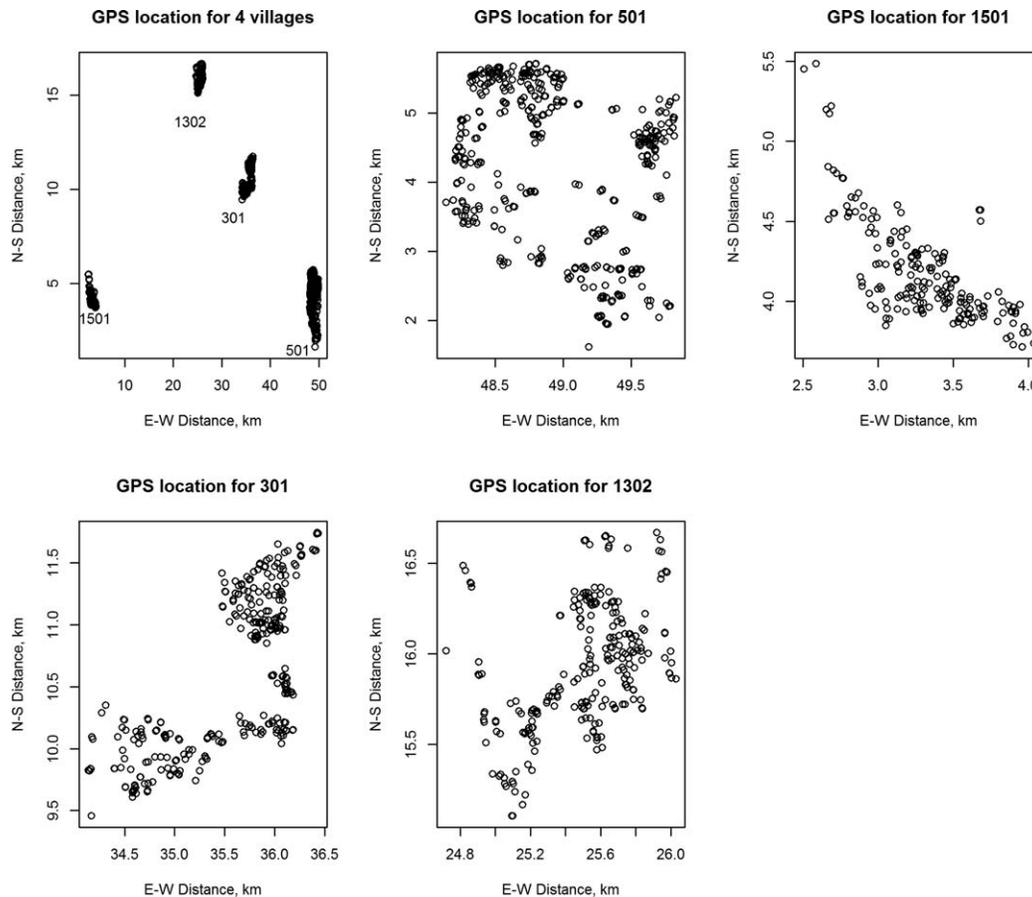
FIGURE 1. Map demonstrating baseline global positioning system (GPS) locations of households in all four villages (*top left*) as well as maps demonstrating GPS location of households in each individual village (501, 1501, 301, 1302).

no significant clustering of either infection or trachoma from 0.30 to 1.00 km.

We also performed a sensitivity analysis comparing the K functions of positive households versus all households to determine if clustering is a function of the comparison group. The results were unchanged. We performed another sensitivity analysis in which we imputed positive and negative data for households where children were missing data. However, the percentage of children missing data was very low (less than 10% at each time point) and the imputations made no difference in the results.

RESULTS

Overall, we studied between 2118 and 2670 children under 10 years of age across the four villages over the study period. The mean age of the children studied was slightly over 4 years. At baseline, 499 children (23.56%) had *C. trachomatis* infection. The number of children infected declined to 114 (5.10%) at 42 months after four rounds of MDA. There was a decline in infection in the periods six months after MDA (6, 18, 30, and 42 months), but there was a slight rebound in infection in periods 12 months after MDA (12, 24, and 36 months).

At baseline there were 597 children (27.74%) with active trachoma, and this number declined to 176 (7.89%) at 42 months. Unlike *C. trachomatis* infection, there was little rebound in active trachoma at 12 months after MDA (12, 24, and 36 months) compared to 6 months after MDA (6, 18, 30, and 42 months), with the only rebound occurring between 6

(12.18% active trachoma) and 12 (16.23% active trachoma) months. Table 1 describes the age, infection, and active trachoma status in the children studied over 42 months.

A total of 918 to 991 households in the 4 villages was included over the 42 months of this analysis. At baseline 306 households (32.59%) had at least one child with *C. trachomatis* infection and at 42 months only 73 households (7.50%) had at least one child with infection. As with the individual data, the household data showed rebound in infection 12 months after MDA compared to 6 months after MDA. A total of 396 households (41.95%) had at least one child with active trachoma at baseline, with a decline to 146 households (15.01%) with at least one child with active trachoma at 42 months. As with individual data, there was only rebound in active trachoma at 12 months. Table 2 demonstrates the amount of *C. trachomatis* infection and active trachoma at the household level.

Figure 1 consists of geospatial plots of all 4 villages together and each individual village. The four villages in this study occupied distinct areas in a 50 km (East-West) by 17 km (North-South) grid. The village occupying the largest geospatial area (501) was approximately 2 km (East-West) by 5 km (North-South) in size, while the smallest village (1302) was 1.2 by 1.5 km in size. The households in the village did not have uniform density over the rectangular area of the plots. There were several specific points of high household density in each of the villages. The median distance between a household and its nearest neighbor was 0.037 km (interquartile range [IQR], 0.017–0.070 km).
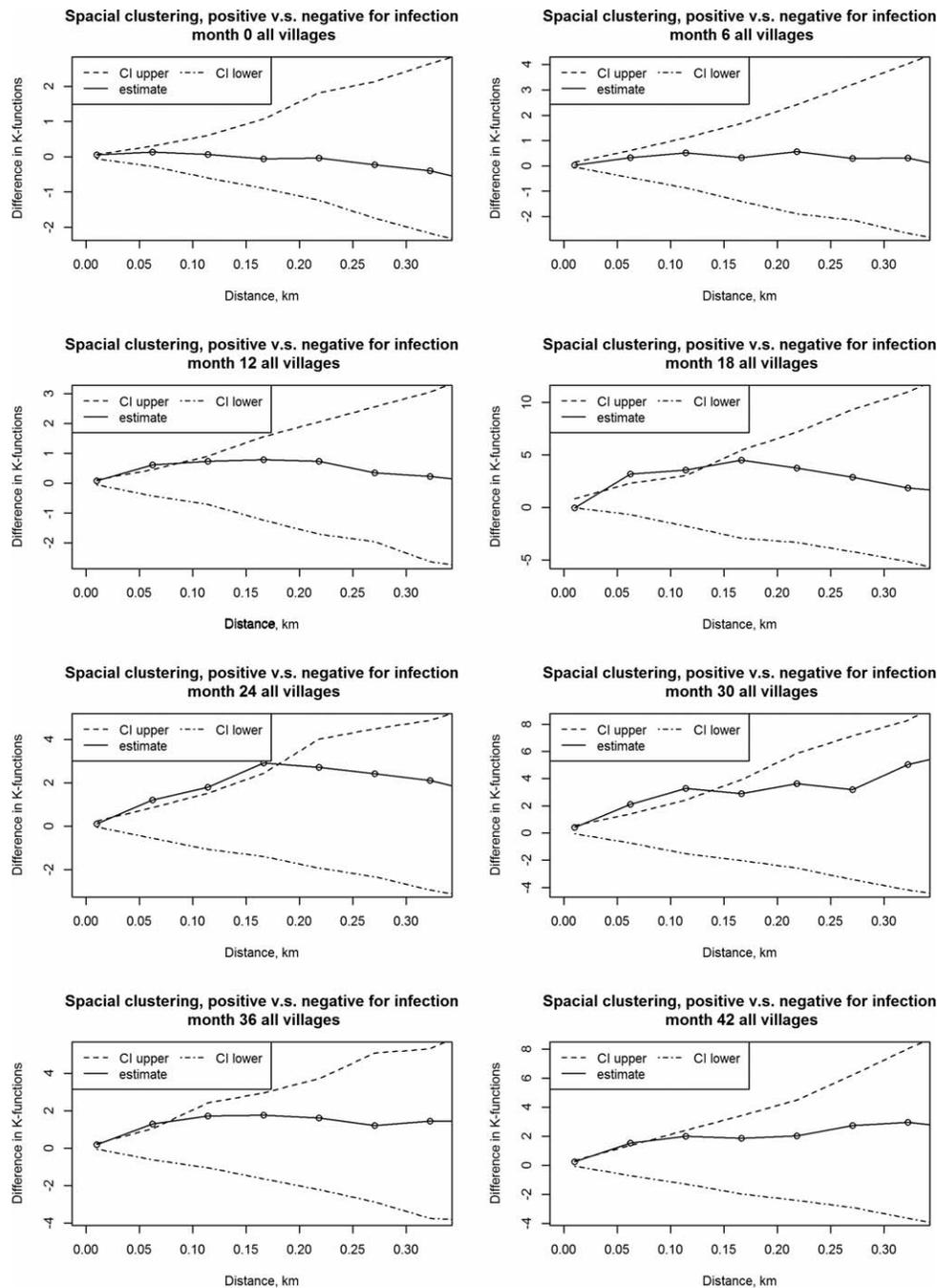
FIGURE 2. K plots for *C. trachomatis* infection from baseline to 42 months. The *solid line* represents the difference in K functions, while the *dotted line* represents the upper and lower confidence intervals generated by 2000 bootstrap iterations. There is significant clustering of *C. trachomatis* infection when the *solid line* falls outside the confidence intervals.

The K plots for households with at least one child with infection are demonstrated in Figure 2. At baseline and 6 months (after one round of MDA), there was no significant clustering of infected households. At 12 months, there was borderline clustering at distances (*d*) between 0 and 0.05 km. At 18 months (after two rounds of MDA) there was evidence of significant clustering of infected households between 0.05 and 0.10 km. At 24 months, the clusters became larger, with clustering occurring between 0.05 and 0.20 km. The size of the clusters declined to between 0.00 and 0.14 km at 30 months and these smaller clusters of infection were seen throughout the remainder of the study at 36 and 42 months.

Figure 4 demonstrates an example of the geographic distribution of infection in a group of households in village 1302 at 12 (Fig. 4A), 18 (Fig. 4B), and 24 (Fig. 4C) months. Here, we see that the number of households infected in a geographic region decreased at 18 months (6 months after prior MDA), but then rebounded at 24 months (12 months after prior MDA). The average number of children with infection per household in Figure 4 decreased from 0.85 at 12 months to 0.28 at 18 months and was 0.50 at 24 months. Figure 4 demonstrates that the spread of infection between 18 and 24 months occurred outwardly from a focus of households that have infection at 18 months.
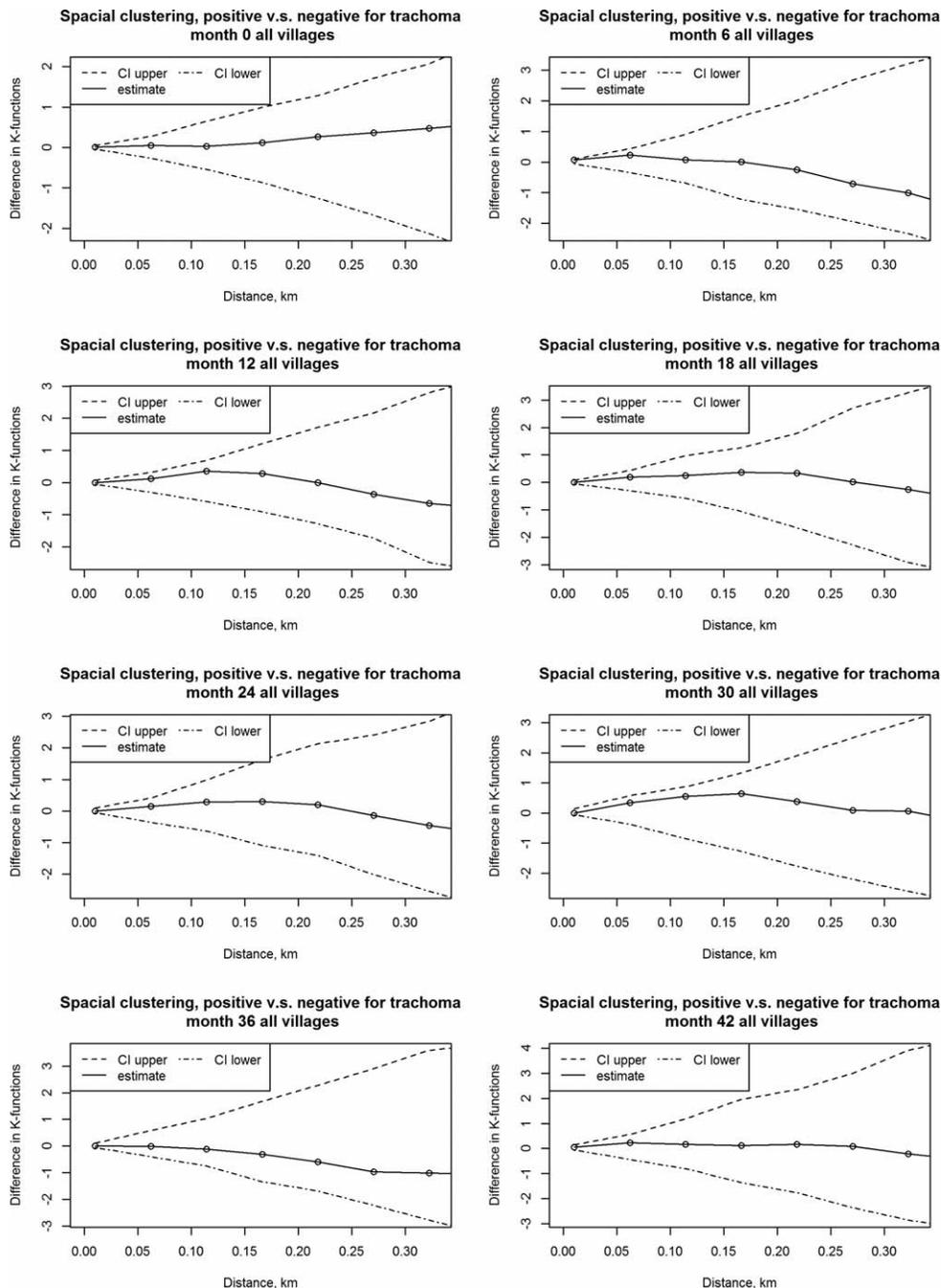
FIGURE 3. K plots for clinical trachoma from baseline to 42 months. The *solid line* represents the difference in K functions, while the *dotted line* represents the upper and lower confidence intervals generated by 2000 bootstrap iterations. There is significant clustering of clinical trachoma when the *solid line* falls outside the confidence intervals.

Figure 3 demonstrates K plots for households with at least one child with active trachoma. Unlike the K plots for infection, the $\hat{D}(d)$ remained within the confidence intervals generated by bootstrapping over all time periods, suggesting that trachoma, unlike infection, does not tend to cluster after MDA, but declines uniformly within the village.

DISCUSSION

In this study, we used spatial analysis to detect clustering of *C. trachomatis* infection and active trachoma in villages under-
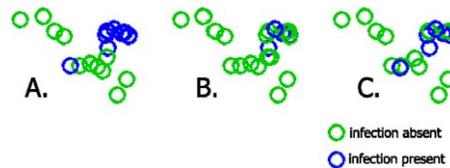
FIGURE 4. The geospatial distribution of infection in a group of households in village 1302 at 12 (**A**), 18 (**B**), and 24 months (**C**). *Green circles* represent households without any infection, while *blue circles* represent households with at least one infected individual.

TABLE 1.   Mean Age, *C. trachomatis* Infection and Active Trachoma in Surveyed Children Over Time

Time	Mean Age (SD)	n of Children Surveyed	Positive C. Infection		Active Trachoma	
			n	%	n	%
Baseline	4.02 (2.79)	2152	499	23.6	597	27.7
6 months	4.03 (2.81)	2119	216	10.2	258	12.2
12 months	4.14 (2.81)	2236	294	13.2	363	16.2
18 months	4.07 (2.84)	2142	84	3.9	296	13.8
24 months	4.17 (2.78)	2310	174	7.5	281	12.2
30 months	4.12 (2.78)	2212	83	3.8	209	9.5
36 months	4.30 (2.76)	2296	143	6.2	225	9.8
42 months	4.21 (2.79)	2234	114	5.1	176	7.9

going annual MDA. Clustering of infection was not seen in these villages at baseline before MDA. The MDA steadily reduced the prevalence of infection at each of the yearly surveys, and following the second round of MDA, infection appeared in significant clusters. These clusters grew at 24 months and thereafter declined in size after successive rounds of MDA.

An earlier study in a single village with higher prevalence demonstrated that clustering of infection was present at baseline before treatment.[10] This difference in baseline clustering could be due to methodologic differences between the two studies. The previous study measured chlamydial load and defined a positive household as having a high mean load of infection, whereas we defined a positive household as having at least one child with infection, based on a positive laboratory test. It is possible that any clustering of high load households was diluted in our study with the inclusion of all children with any infection. We did not measure load of infection and so cannot try to analyze our data the same way. It also is possible that the previous village differed in other ways from the villages in this study; with a higher prevalence of infection, transmission among neighboring households may have been easier, leading to evidence of clustering at baseline.

We found that infection begins to cluster after MDA, and this was similar to the previous study on clustering.[10] Other research has found that those with the highest infectious load were most likely still to have infection after MDA,[15] so that the clusters we observed after MDA were likely to be the ones with highest loads before MDA. We observed significant clustering at 18 months, 6 months after the second round of treatment. Clustering tended to occur in a small geospatial area with maximum cluster size < 0.1 km. The median distance between a household and its nearest neighboring household in these villages was 0.037 km (IQR, 0.017–0.070 km), suggesting that the clusters of infection that emerged after the two treatment

rounds consisted of foci of one household and its immediate neighboring households. It is interesting to note that infection must drop dramatically from 23.56% to 3.92% prevalence for the first evidence of clustering to emerge, and is consistent with reducing any infection to just those with high loads, which do tend to cluster. This is supported further by the evidence at 24 months that the clusters had grown in size as infection increased. The increase in cluster size suggests that the initial foci of infection seen at 18 months (infected household and nearest neighbors) spread outwardly to secondary neighbors and even tertiary or quaternary neighbors. After the next round of MDA, however, the clusters decreased in size and stayed constant until 42 months, suggesting the start of control of transmission. One potential source of clustering of infection after MDA is difference in treatment compliance based on treatment regimen. For instance, children under 6 months who received topical tetracycline, may have been relatively poorly compliant with treatment compared to those 6 months or older who received oral azithromycin. Such a difference in treatment compliance can lead to the clustering and rebound of infection we observed after MDA. However, we have shown previously, in this dataset, that children under 6 months given topical tetracycline are not a source of re-emergent infection for other children in their households. They also are not more likely to have infection at the next visit.[16]

Of note, active trachoma did not cluster during any of the time points studied. Since active trachoma takes longer to resolve after infection, it is reasonable that active trachoma did not cluster and infection did. One prior cross-sectional study in a setting of higher prevalence of disease demonstrated clustering of trachoma.[11] However, the study population consisted of children and adults. Additionally, the investigators assessed clustering using the Kulldorf spatial scan statistic, which identifies local areas of clustering, and unlike the K

TABLE 2.   *C. trachomatis* Infection, Active Trachoma and Mean Number of Children in Surveyed Households Over Time

Time	n of Households Surveyed	With at Least 1 Child With *C. trachomatis* Infection		With at Least 1 Child With Active Trachoma		Mean n of Children per Household (SE)
		n	%	n	%	
Baseline	944	306	32.6	396	42.0	2.3 (1.2)
6 months	919	146	15.9	193	21.0	2.3 (1.2)
12 months	966	183	18.9	257	26.6	2.4 (1.1)
18 months	927	50	5.4	223	24.1	2.4 (1.2)
24 months	988	107	10.8	209	21.2	2.4 (1.1)
30 months	972	60	6.2	162	16.7	2.4 (1.1)
36 months	991	92	9.3	172	17.4	2.4 (1.1)
42 months	973	73	7.5	146	15.0	2.5 (1.1)

function, does not provide a global statistical measure of clustering.[17] A later longitudinal study in another high prevalence setting, which used K function analysis, showed no clustering of trachoma, before or after treatment.[10]

There are several limitations to this study. First, the four villages in this study are not geographically isolated from the rest of Kongwa district and may have had newcomers with or without infection entering at various time points into various geospatial locations. We attempted to capture migrants by performing census updates on a yearly basis alongside MDA. However, any migrants entering or leaving the villages six months after MDA were not captured until the next MDA. These newcomers may dilute or enhance the effects of clustering we demonstrated six months after treatment. Second, the Amplicor test we used, although sensitive in detecting infection, may not be the best indicator of transmission potential. Since clustering of infectious disease depends on potential for transmission, a test that captures transmission potential, such as infectious load, may be more suitable. Finally, we had a small number of children with missing infection and active trachoma data (<10% missing at each time point). We accounted for this by imputing these missing values as all positive or all negative. The results we obtained using the nonimputed analysis were virtually identical to the imputed analysis.

In conclusion, the results offer new insight into the spatial distribution of *C. trachomatis* infection in communities undergoing several rounds of mass treatment. This study demonstrated that *C. trachomatis* infection does start to cluster after multiple rounds of MDA. Such clusters of infection may increase in size if the annual antibiotic pressure is removed, although the absence of growth after the three rounds suggests the start of control of transmission. Unfortunately, these clusters are not able to be detected using the active signs of trachoma, which did not cluster. This may argue for the use of a rapid test in the field that can detect the cluster of infection during surveys, and treat the household and surrounding households.

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