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Ocular *Chlamydia trachomatis* Infection Under the Surgery, Antibiotics, Facial Cleanliness, and Environmental Improvement Strategy in Amhara, Ethiopia, 2011–2015

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Background. World Health Organization (WHO) recommendations for starting and stopping mass antibiotic distributions are based on a clinical sign of trachoma, which is indirectly related to actual infection with the causative agent, *Chlamydia trachomatis*.

Methods. This study aimed to understand the effect of SAFE (surgery, antibiotics, facial cleanliness, and environmental improvement) interventions on ocular chlamydia in Amhara, Ethiopia, by describing the infection prevalence in a population-based sample of children aged 1–5 years. Trachoma surveys were conducted in all districts of Amhara, from 2011 to 2015 following approximately 5 years of SAFE. Ocular swabs were collected from randomly selected children to estimate the zonal prevalence of chlamydial infection. The Abbott RealTime polymerase chain reaction assay was used to detect *C. trachomatis* DNA.

Results. A total of 15 632 samples were collected across 10 zones of Amhara. The prevalence of chlamydial infection in children aged 1–5 years was 5.7% (95% confidence interval, 4.2%–7.3%; zonal range, 1.0%–18.5%). Chlamydial infection and trachomatous inflammation–intense (TI) among children aged 1–9 years were highly correlated at the zonal level (Spearman correlation [r] = 0.93; $P < .001$), while chlamydial infection and trachomatous inflammation–follicular were moderately correlated ($r = 0.57$; $P = .084$).

Conclusions. After 5 years of SAFE, there is appreciable chlamydial infection in children aged 1–5 years, indicating that transmission has not been interrupted and that interventions should continue. The sign TI was highly correlated with chlamydial infection and can be used as a proxy indicator of infection.

Keywords. trachoma; *Chlamydia trachomatis*; Ethiopia; survey.

National trachoma control programs, following World Health Organization (WHO) recommendations for starting and stopping mass antibiotic distributions for trachoma, base programmatic decisions on the measurement of trachomatous inflammation–follicular (TF) among children aged 1–9 years through a visual examination of the ocular conjunctivae [1]. However, at the individual level, this indicator does not correlate well with infection prevalence of *Chlamydia trachomatis*, the etiological bacterium of trachoma, particularly after mass drug administration (MDA) with azithromycin or topical tetracycline [2]. This is thought to be due, in part, to the natural history of the infection itself whereby the duration of infection and the duration of clinical signs do not completely overlap [3]. At the community level, the correlation between TF and chlamydial infection is higher than that seen at the individual level, but appears to weaken or disappear in post-MDA settings [2, 4]. To date, fewer studies have measured the prevalence of chlamydial infection alongside the clinical signs TF and trachomatous inflammation–intense (TI) at the district level or higher in programmatic, post-MDA settings [5, 6].

An initial blindness and low vision survey conducted in Ethiopia in 2006 demonstrated that Amhara was the worst trachoma-affected region in the country [7]. Early estimates in pilot districts within the region ranged from 49% to 90% TF [5]. These data triggered a zonal-level baseline survey, which demonstrated a high prevalence of TF region-wide; thus, a full scale-up of the WHO-recommended SAFE strategy (surgery, antibiotics, facial cleanliness, and environmental improvement), including 3–5 annual rounds of district-wide MDA, was warranted [8]. Because programmatic scale-up took a number of years to reach all districts, 6 survey rounds, conducted between 2011 and 2015, were needed to assess the impact of SAFE interventions. To inform programmatic decision making, the Amhara trachoma control program collected conjunctival swabs from a population-based sample of children 1–5 years of age from each district surveyed. The aim of this study was to

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determine the region-wide prevalence of chlamydial infection after 5 years of SAFE. Furthermore, chlamydial infection prevalence was compared to the prevalence of TF and TI to help elucidate the relationships between these indicators at this scale.

METHODS

Ethics Statement

All survey methods were reviewed and approved by the Amhara Regional Health Bureau, Ethiopia, and by the Emory University Institutional Review Board under protocol 079-2006. All participants provided informed verbal consent as illiteracy was high throughout the region. Consent was first obtained from heads of households, followed by individual participants, and then assent for minor participants according to the principles of the Declaration of Helsinki.

Survey Design

Population-based trachoma impact surveys were conducted in all districts of the Amhara region (150 districts), except for 2 urban centers, to assess trachoma clinical signs [9]. Nearly all districts (147/150 [98%]) had received at least 5 years of annual MDA (range, 3–7 years). The methodology of these surveys has been detailed elsewhere [9]. In brief, a multistage cluster randomized methodology was used, whereby villages were selected using population proportional to estimated size and, within a village, a modified segmentation approach was used to select households. The number of villages chosen per district, and thus per zone, was proportional to district population size. All individuals living in all households within selected segments were enumerated and all present and consenting household members were examined for clinical signs of trachoma.

During the cluster selection process, every other cluster was chosen for conjunctival swab collection (Supplementary Figure 1). To determine the zonal prevalence of ocular *C. trachomatis* among children aged 1–5 years, 1107 children were needed per zone, which assumes a prevalence level of 4% ± 2%, α error of .05, and a design effect of 3.0. Assuming a 10% nonresponse rate, at least 1217 children 1–5 years of age per zone were targeted for swab collection. The first 25 children in this age range encountered by survey teams from selected households within each cluster were selected. If 2 children in 1 household were in this age range, survey software randomly selected 1 child to be offered the chance to participate.

Clinical Training and Examination

Trachoma graders were integrated eye care workers who participated in a week-long intensive training before each survey round to continually ensure standardization with expert graders, and to prevent grading drift. During training, graders first needed to pass a photographic slide examination and then a field reliability examination, where they graded children for the clinical signs of trachoma. Their grades were then compared to the consensus grade of 3 expert trachoma graders as the gold standard. Graders with an interobserver agreement of at least 84% for TF and $\kappa \geq 0.7$ proceeded to the field [9]. Consenting survey participants had their eyes examined for the signs of trachoma using the WHO simplified grading scheme [10]. Examination was conducted using a binocular loupe with 2.5× magnification and adequate light, including a flashlight if necessary. Individuals diagnosed with TF and/or TI were immediately offered treatment per current WHO guidelines.

Conjunctival Swab Collection

Graders put on powder-free latex gloves prior to everting the participant's eyelid and swabbing, and a new pair of gloves was used for every participant. A designated "tuber," wearing gloves, aseptically removed the polyester-tipped swab (Fisher Scientific) from the packet to aid the grader in the collection process. The grader then swabbed the upper tarsal conjunctiva firmly 3 times, rotating 120 degrees along the swab's axis at each pass to collect a sufficient epithelial specimen [11]. With the help of the tuber, swabs were placed into a 2.0-mL Nunc tube, labeled, and held/transferred to the laboratory in a cooler bag with ice packs. Swabs were stored at –20°C until tested. To assess field contamination, a sterile dry swab was passed within 1 inch of the conjunctiva from a randomly chosen 5% of monitored children [11]. These negative field control "air swabs" were transported with the study samples.

Laboratory Assessment and Quality Control

All diagnostic testing of specimens was performed at the Amhara Public Health Research Institute in Bahir Dar, Ethiopia. Conjunctival swabs from each district were randomized and 5-pooled to conserve time and resources using a protocol developed for previous randomized cluster trials conducted in Amhara [4, 11, 12]. "Air swabs" were also pooled before testing. Pools were created by rehydrating the swabs with 1.0 mL of molecular-grade water. Then 100-µL aliquots from 5 swab specimens were combined into a sterile tube (total volume = 500 µL) for testing. Pools were processed with the RealTime (Abbott Molecular, Des Plaines, Illinois) polymerase chain reaction (PCR) assay to detect *C. trachomatis* DNA, using the automated Abbott m2000 System. The RealTime assay targets 2 highly conserved targets on the cryptic plasmid of *C. trachomatis* and has internal control primers that will detect the presence of PCR inhibitors in the sample [13]. Each run contains 1 negative and 2 positive calibrators and a processing control. The decision cycle for a positive result was determined using the average of the 2 positive calibrators plus a predetermined number of cycles. Pools with equivocal results were retested, and if still equivocal, then the individual samples in the pool were tested. When a district pooled prevalence was >80%, samples from that district were repooled into pools of 3 for greater accuracy [12]. After initial assays, 5 of 150 (3.3%) districts required repooling.

Strict laboratory quality control (QC) procedures were maintained throughout the project. The laboratory was monitored

monthly for amplicon contamination. Each quarter, QC panels consisting of 20 positive/negative chlamydia samples prepared by the University of California, San Francisco (UCSF) were tested masked. Additionally, the m2000 instruments were routinely serviced by an Abbott technician. For assay performance, 10% of assayed pools (half positive, half negative) were sent masked to UCSF to handle the retesting of samples. To assess the false-negative rate, 100 random negative pools were deconstructed and the individual samples were tested. Laboratory technicians were masked to the trachoma clinical outcome of participants, the district of residence, or whether a sample was a survey swab or a control swab.

Statistical Analysis

Infection for this report was defined as present if *C. trachomatis* DNA was detected in a pool using PCR. District chlamydial infection prevalence was estimated from the district pooled prevalence as the number of positive individual samples most likely to have resulted in observed pooled results [4, 11, 12]. Zonal prevalence estimates were weighted by the number of pools per district, and robust confidence intervals (CIs) were estimated using Taylor-series linearization. Zonal estimates of TF (with/without TI) and TI (with/without TF) were weighted using selection probabilities and estimated using survey procedures accounting for the multilevel nature of the survey design. Survey data also included children's age and sex. Spearman correlations were calculated comparing TF (with/without TI) and TI (with/without TF) with chlamydial infection. Normally distributed data were described using the mean and 95% CI. Nonnormally distributed data were described by the median and interquartile range (IQR). Linear regression was used to obtain the explained variance in chlamydial infection. All analyses were conducted using Stata 13.1 software (StataCorp, College Station, Texas).

RESULTS

Between 2011 and 2015, a total of 15 632 conjunctival swabs were collected from children aged 1–5 years in 770 villages across 150 districts in 10 zones representing the entire region of Amhara. Samples were pooled into 3427 pools for processing. The median number of clusters per district was 5 (IQR, 3–6), the median number of swabs per cluster was 21 (IQR, 17–25), and the median number of samples per district was 97 samples (IQR, 66–126). Ninety-five percent of districts had either 5 (65%) or 6 (30%) rounds of annual MDA, the median time since last MDA was 8 months (IQR, 8–8), and 53% of swabs were collected in the dry season (September–April). The ocular swab sample was 51.6% female and the mean age was 3.3 years. The age–sex distribution among the swabbed sample was similar to the distribution of the survey population overall (Supplementary Table 1).

Standard QC results showed 100% (20/20) for testing quarterly control panels and 0–5% contamination from routine amplicon monitoring (20 swipes/month). For external QC tested pools, there was 98% (332/339) agreement. Pools with discordant results were low-level positives (delta cycle range, 0.08–3.37). For specificity testing, a further set of 100 random negative pools were unpooled and run individually (n = 428). There was 1 positive individual sample, indicating a 99.8% (427/428) specificity, which is better than the performance stated in the RealTime package insert. For field control "air swabs," there was 1 positive pool. A retest of the individual specimens in this pool found only 1 positive sample. Thus, 0.1% (1/762) of air swabs was contaminated.

The prevalence of chlamydial infection in children aged 1–5 years in Amhara region was 5.7% (95% CI, 4.2%–7.3%). Four zones had a prevalence of <5%, 4 had a prevalence between 5% and 10%, and 1 was >10% (Figure 1). Zonal point estimates ranged from a low of 1.0% in both Awi and Oromia zones to a high of 18.5% in Waghemra zone (Table 1).

A total of 68 959 children aged 1–9 years were examined for the clinical signs of trachoma from 1664 communities during the surveys conducted between 2011 and 2015. The prevalence of TF in this age group was 26.2% (95% CI, 25.2%–27.2%; zonal range, 16.8%–54.7%), while the regional prevalence of TI was 5.6% (95% CI, 5.2%–6.0%; zonal range, 3.4%–13.7%). In all 10 zones, the prevalence of TF was considerably higher than the prevalence of both TI and chlamydial infection (Figure 2). Conversely, the prevalence point estimates of TI and chlamydial infection were similar. Furthermore, chlamydial infection and TI were highly correlated at the zonal level (Spearman correlation [r] = 0.93; $P < .001$), while infection and TF were moderately correlated ($r = 0.57$; $P = .084$) (Figure 3A and 3B). These correlations were of similar magnitude when chlamydial infection was compared to TF ($r = 0.67$; $P = .03$) and TI ($r = 0.86$; $P = .001$) in children aged 1–5 years. In a regression model, TI explained nearly 64% of the variance in chlamydial infection ($R^2 = 0.64$), and the addition of TF to the model did not increase the R^2 considerably ($R^2 = 0.66$).

At the district level (n = 150), chlamydial infection prevalence ranged from 0.0% to 38.3% and the distribution was skewed to the right (median, 1.3%; mean, 4.9%). All 10 zones had at least 1 district where no chlamydial infection was detected, and no infection was detected within the 21 districts with a TF prevalence <10% (Figure 4). At the district level, median chlamydial infection prevalence increased with each commonly used category of TF prevalence [1]. The prevalence of chlamydial infection did not differ by season in which ocular swabs were collected (dry season, 6.9% [95% CI, 4.2%–9.5%]; wet season, 4.8% [95% CI, 3.1%–6.4%]), and infection prevalence did not increase with time since MDA (7 months: 2.0% [95% CI, 0.2%–3.8%]; 8 months: 6.9% [95% CI, 5.0%–8.8%]; 9 months: 2.8% [95% CI, 0.5%–5.1%]; $P_{trend} = .222$). Last, no apparent trends were observed when comparing chlamydial infection prevalence across year of swab collection, (5.8%, 2.0%, 6.7%, 8.2%,

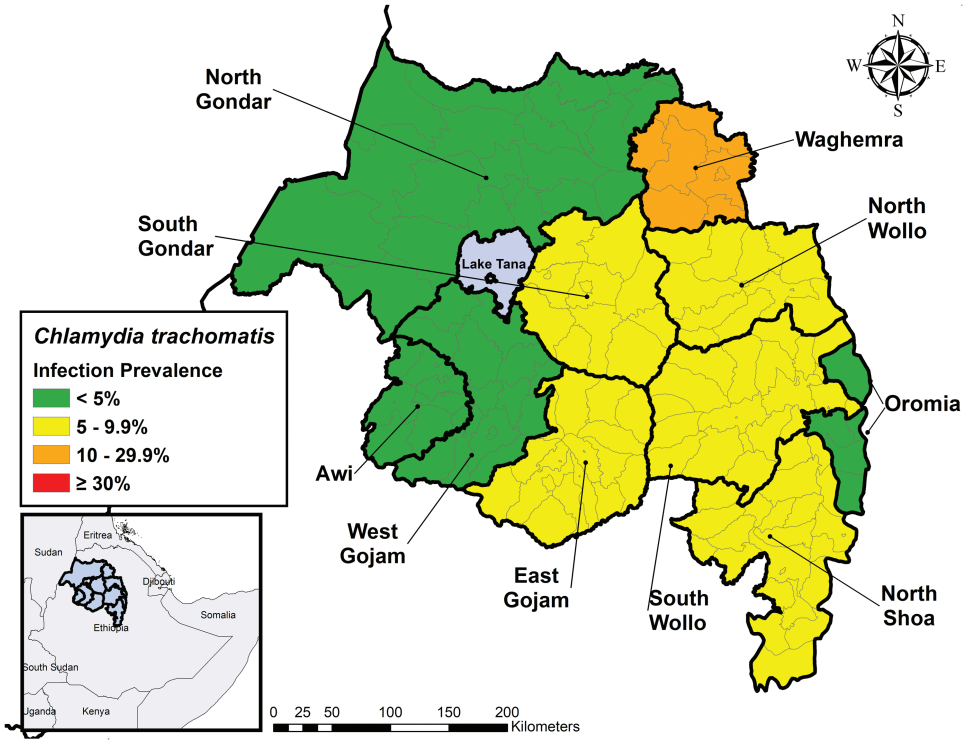

Figure 1. Geographical distribution of chlamydial infection prevalence among children aged 1–5 years in Amhara, Ethiopia, 2011–2015.

and 2.8% in 2011, 2012, 2013, 2014, and 2015, respectively; $P_{trend} = .574$).

DISCUSSION

This study demonstrated that monitoring ocular chlamydial infection at a programmatic scale was possible. Chlamydial infection prevalence in Amhara was 5.7% (zonal range, 1%–18.5%) among children aged 1–5 years after an average of 5 annual rounds of MDA. All zones had districts where no chlamydial infection was detected, while some districts had high levels of infection despite the amount of antibiotic distributed. While the prevalence of chlamydial infection was moderately correlated with TF at the zonal level, infection prevalence estimates were both highly correlated with TI, and of similar magnitude as TI prevalence estimates, suggesting that TI is a robust indicator of the level of chlamydial infection in an administrative zone.

Community-wide treatment with azithromycin is effective in reducing the prevalence of chlamydial infection, even in hyperendemic communities [11, 14–17]. Randomized trials

Table 1. Sample Size and Zonal Prevalence of Chlamydial Infection Among Children Aged 1–5 Years by Administrative Zone, Amhara, Ethiopia, 2011–2015

Zone	No. of Districts	No. of Clusters	No. of Pools	No. of Swabs	Chlamydial Infection Prevalence, % (95% CI)	District Prevalence Range, %, Min–Max
Oromia	7	25	117	478	1.0 (0–2.1)	0–3.7
South Wollo	22	119	490	2245	5.5 (.9–10.2)	0–27.3
North Wollo	12	73	299	1286	6.7 (2.8–10.6)	0–20.6
North Shoa	24	99	372	1579	8.3 (4.1–12.6)	0–33.9
Waghemra	7	28	150	587	18.5 (6.0–30.9)	0–31.1
Awi	11	45	204	908	1.0 (.1–1.9)	0–3.2
North Gondar	23	117	545	2622	2.1 (.3–3.8)	0–17.0
West Gojjam	16	75	362	1695	2.5 (.0–5.0)	0–15.3
South Gondar	10	99	488	2407	5.8 (2.7–8.9)	0–11.9
East Gojjam	18	90	400	1825	9.8 (4.3–15.3)	0–38.3
Total	150	770	3427	15632	5.7 (4.2–7.3)	0–38.3

Abbreviation: CI, confidence interval.

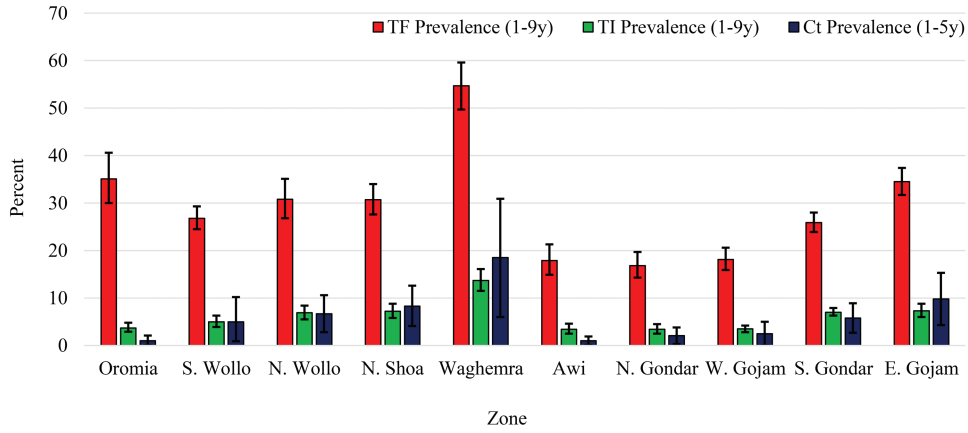

Figure 2. Zone-level prevalence of chlamydial infection (Ct), trachomatous inflammation–follicular (TF), and trachomatous inflammation–intense (TI) in Amhara, Ethiopia, 2011–2015.

conducted in Ethiopia have demonstrated that both annual and biannual MDA treatment strategies could reduce the prevalence of infection drastically [11, 15, 18]. Although infection data were not available in Amhara prior to the introduction of MDA, infection was most likely substantial, as early surveys demonstrated TF prevalences as high as 90% [5, 8]. Available pre-SAFE chlamydial infection data from a trial conducted in Amhara demonstrated high levels of infection in children, between 38.3% and 41.9% [11]. After 4 years of annual treatment, infection in those study communities was reduced from 41.9% to 1.9%. This current study demonstrated that among a population-based sample of children born largely in a time encompassed by the SAFE strategy, including an average of 5 annual doses of MDA, chlamydial infection regionwide was 5% and was not detected in many districts. SAFE interventions have most likely had a significant impact on chlamydia transmission in Amhara. As the prevalence of TF tends to lag the prevalence of chlamydial infection within communities, Amhara may be on a trajectory to reach elimination thresholds based on TF, although it may take more years than current WHO treatment guidelines suggest [1, 19].

The role that chlamydial infection monitoring should play in programmatic decisions is still not clear. This is partially because it is not yet known whether or not there is a minimum level from which infection will not recrudesce in a population. Several longitudinal studies have demonstrated that infection can return from low levels, at least in hyperendemic areas [15, 16, 20, 21]. In Ethiopia, antibiotic treatment reduced chlamydial infection from 63.5% at baseline to 2.6% after 4 biannual treatments [15]. However, after stopping MDA, the prevalence of infection returned to 25.2% after 18 months. These results suggest rapid reinfection from nearby untreated areas if the only trachoma intervention is antibiotic and environmental conditions that favor high transmission are not altered. Two zones in Amhara had a chlamydial infection prevalence as low as 1%; however, in both, the TF prevalence remained high enough to warrant MDA for another 3–5 years. These additional rounds may keep infection prevalence low or even eliminate it from these zones. It remains to be seen whether recrudescence will take place when all neighboring districts have been under antibiotic pressure and there have been considerable improvements in hygiene behavior and environmental cleanliness [5]. Elimination of chlamydial infection has been demonstrated in formerly hyperendemic areas at the community and district level and, although difficult, it may be more achievable than first thought [6, 14, 22, 23].

The correlation between chlamydial infection and the clinical signs of trachoma appear to depend on whether an area has had MDA [2, 4]. This may be owing to a quick resolution of infection post-MDA, but a lagging resolution of TF over a period of weeks or months possibly owing to local inflammation [3]. A recent meta-analysis of population-based estimates demonstrated that pre-MDA, infection and TF were strongly correlated (r = .92), while post-MDA, the 2 measures were only moderately correlated (r = .60), and that TF tends to overestimate infection [2]. In Amhara, a post-MDA setting, the correlation between chlamydial infection and TF was also moderate (r = .57) and TF prevalence overestimated infection in each zone regardless of the infection prevalence. Furthermore, no districts with TF <10% had detectable infection. Monitoring programmatic progress with signs of TF alone in a post-MDA setting is problematic given this tendency for consistent overestimation. Individuals with TI are more likely to have infection, particularly infection with high chlamydial loads [24]. The correlation between chlamydial infection and TI in Amhara was high (r = 0.93), and stronger than that reported in the recent meta-analysis of 8 studies and approximately 11 000 participants (r = 0.50) [2]. These findings are difficult to compare as the meta-analysis consisted of studies which were population-based at the village or community level. Given wide community-to-community variation in infection, population-based

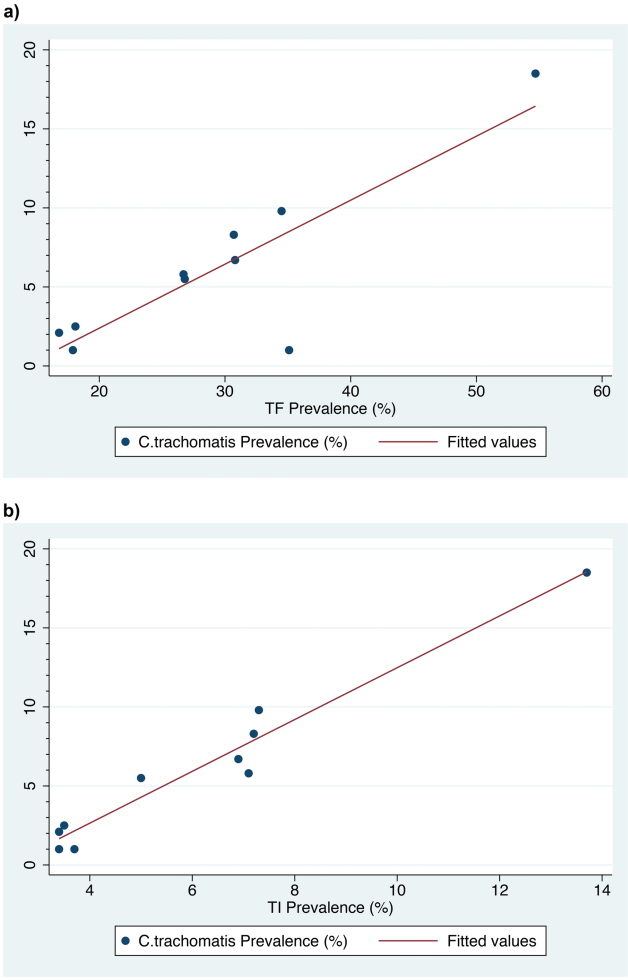

Figure 3. Scatterplot between zone-level prevalence of chlamydial infection (age 1–5 years) and trachomatous inflammation–follicular (TF; *A*) (age 1–9 years) and trachomatous inflammation–intense (TI; *B*) (age 1–9 years), Amhara, Ethiopia, 2011–2015.

estimates from the district or higher are likely more informative to control programs [11, 15]. To date, TI has not gained traction as a programmatic indicator. The Amhara experience suggests that TI among children aged 1–9 years is a robust indicator of population levels of chlamydial infection.

This study had several limitations. The stability of specimens stored in a −20°C freezer beyond the stated manufacturer's storage conditions is not known. However, the degradation of DNA at −20°C would have been minimal compared to samples held at 4°C or room temperature where DNA samples would show varying degrees of evaporation. Bacterial DNA has been shown to remain detectable by PCR over time under various conditions, and an infection prevalence-by-year-collected trend was not detected in these data [25, 26]. It should be noted that the RealTime assay will detect both nonviable and viable chlamydia. For the latter, homebrew nucleic acid amplification tests have been developed to detect ribosomal RNA, which is an indicator of biological activity, and may be helpful in monitoring MDA. However, these field tests are difficult to perform and would pose logistical challenges to programs. In addition, high omp1 DNA (>10^5 copies/swab) has been shown to be associated with high 16S ribosomal RNA (>10^5 copies/swab) [27]. Pooling samples may have led to dilution and a higher chance of false negatives [6]. However, our QC results had a 99.8% (427/428) specificity and equivocal pools were run individually.

Despite these limitations, the assay used to detect chlamydial DNA is widely used to detect genital infections. Previous studies have shown RealTime to be highly sensitive (95.3%) and specific (99.9%) for the diagnosis of *C. trachomatis* from urine and ocular swabs [13, 28, 29]. The RealTime assay has compared favorably to other nucleic acid amplification tests for detecting *C. trachomatis* DNA and RNA commonly used in trachoma research [13, 28, 30]. Furthermore, the m2000 platform allows for testing many samples at 1 time, particularly if pooled, which is ideal for large population-based and cluster-randomized studies.

From 2011 to 2015, the trachoma control program of Amhara, Ethiopia, collected >15 000 samples from children aged 1–5 years to monitor the impact of SAFE under current treatment guidelines. Chlamydial infection has likely decreased greatly since the beginning of the SAFE strategy; however, given current levels, additional years of intervention will be needed. In lieu of infection monitoring, control programs serving hyperendemic areas should consider TI as an indicator of chlamydial infection at the district level or higher.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

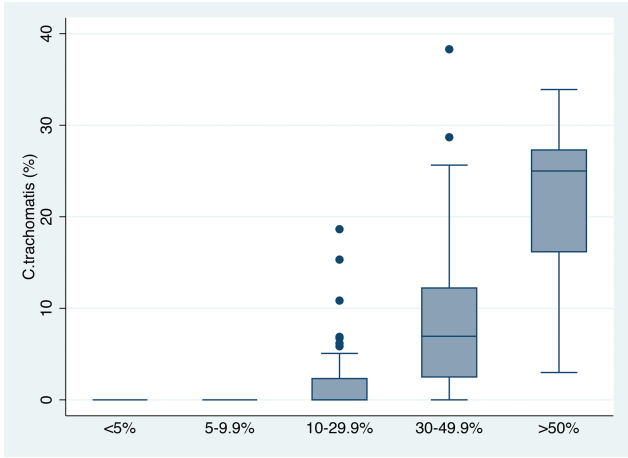

Figure 4. District-level prevalence of chlamydial infection among children aged 1–5 years by trachomatous inflammation–follicular categories in Amhara, Ethiopia, 2011–2015.

Notes

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