Trachoma prevalence remains below threshold in five districts after stopping mass drug administration: results of five surveillance surveys within a hyperendemic setting in Amhara, Ethiopia

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**Background:** The World Health Organization (WHO) recommends conducting trachoma surveillance surveys in districts where the elimination targets have been met and following a minimum 2-year period after cessation of mass drug administration (MDA) in order to determine the sustainability of low trachoma levels.

**Methods:** In 2015, population-based surveillance surveys were conducted in five districts of Amhara, Ethiopia. All five districts had a prior trachomatous inflammation–follicular (TF) prevalence among children 1–9 y of age of <5% determined by an impact survey and had not received MDA for ≥2 y. Surveys included examinations for trachoma clinical signs and conjunctival swabbing to determine Chlamydia trachomatis infection prevalence.

**Results:** Approximately 1000 children 1–9 y of age were examined for TF and 200 children 1–5 y of age were swabbed per district. All five surveillance districts had a TF prevalence of <5% and infection was only detected in one district. The prevalence of trachomatous trichiasis in adults ≥15 y of age was ≥1% in all districts.

**Conclusions:** In a trachoma hyperendemic region, a TF prevalence <5% was successfully maintained in five districts for ≥2 years after stopping MDA. MDA is still not warranted for these districts, however, the S, F and E components of the SAFE strategy should continue.

**Keywords:** Chlamydia trachomatis, neglected tropical diseases, surveillance, surveys, trachoma

**Introduction**

The global trachoma control program has demonstrated success in recent years with the scale-up of the World Health Organization (WHO) recommended SAFE strategy (surgery for trichiasis to prevent blindness, antibiotics to clear infection, facial cleanliness to reduce transmission and environmental improvement to reduce the risk of transmission). The full SAFE strategy, including annual community-wide mass drug administration (MDA) with antibiotics, is warranted for administrative districts until trachoma impact surveys demonstrate that the prevalence of trachomatous inflammation–follicular (TF) among children 1–9 y of age falls below the elimination as a public health problem threshold of <5% and the prevalence of trachomatous trichiasis (TT) falls below the elimination threshold of <0.2% in adults ≥15 y of age. Early baseline surveys conducted in Amhara, Ethiopia demonstrated that the entire region was hyperendemic for trachoma. Based on these data, the SAFE strategy was scaled-up regionwide starting in 2007. After 5 years of the SAFE strategy, 94% of districts in Amhara remained above the TF elimination threshold. However, some districts had a TF prevalence below this threshold despite being surrounded by districts with a high prevalence of TF. Trachoma control programs are required to conduct population-based surveillance surveys in formerly endemic districts after a period of at least 2 y since the
last round of MDA to ensure trachoma has not re-emerged. However, published surveillance reports to date have come from districts located in trachoma hypo-endemic settings. Less surveillance data are available from formerly trachoma hyper-endemic districts located in largely endemic geographic areas, where the risk of re-emergence after stopping MDA may be a concern. In 2015, as per the WHO recommendations, surveillance surveys were conducted in five such districts with the primary aim of determining whether these districts remained below the 5% TF threshold despite the high trachoma burden in the Amhara region as a whole. Secondary aims of these surveys were to estimate the prevalence of TT in adults and to estimate the prevalence of household access to water and sanitation to inform the trachoma control program of the work remaining in the S, F and E arms of the SAFE strategy.

Methods

Throughout Amhara, trachoma impact surveys were conducted at the district level, defined as the normal administrative unit for health care and known locally as a woreda. The Chilga and Finot Selam districts completed five annual MDA rounds in November 2011, were surveyed for impact in June 2012 and were found to have a TF prevalence among children 1–9 y of age of 4.1% and 0.0%, respectively. These two districts were surveyed again in June 2015, 3.5 years since the last round of MDA (Figure 1). The Gende Wuha, Injibara and Tach Armachiho districts completed five annual MDA rounds in November 2012, were surveyed for impact in June 2013 and were found to have a TF prevalence among children 1–9 y of age of 2.9%, 1.6%, and 2.5%, respectively. These districts were surveyed again in June 2015, 2.5 y after the last MDA. Between the impact surveys and the surveillance surveys, surgical interventions continued in all five districts.

For surveillance survey sample size calculations, a TF prevalence among children 1–9 y of age of 3 ± 2% was assumed, with a design effect of 3.04 (based on previous survey data) and 15% non-response, requiring a total of 977 children. According to previous impact surveys, children 1–9 y of age make up 27% of the population in Amhara and the average household size is 4.1 persons, therefore the survey targeted 16 clusters of 60 households to achieve the desired sample size.

The five population-based surveillance surveys were conducted between June 2015 and August 2015. Cluster selection was conducted in the 5 districts using a multistage cluster randomized selection methodology. In the first stage, villages were selected using the probability proportional to the estimated size approach from a geographically ordered list of villages within each district. In the second stage, a modified segmentation approach was used to select households within villages. All individuals within each selected household were eligible for the survey. Households not at home or refusing to participate were not replaced. The exact same two-stage sampling methodology was used in the 2012/2013 impact surveys. However, in the 2012/2013 surveys, sample size determinations were based on the relative size of each district throughout Amhara.

Training

All graders participated in a 7-d training that included class-based practice and several days of field-based practice whereby

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Figure 1. The prevalence of TF in select zones of Amhara, Ethiopia in 2015 with surveillance districts highlighted.
trainees graded children under the supervision of trainers. Graders were from a pool of integrated eye care workers, who are government health care workers in Ethiopia and are trained TT surgeons. In order to participate in the survey, graders had to pass a slide reliability test of 50 standardized conjunctival images as well as a field-based reliability exam of 50 eyes, whereby their score was compared to the consensus grade of three expert grader trainers (an ophthalmologist, a cataract surgeon and the regional trachoma director). All graders were required to achieve a minimum score of 84% agreement (κ=0.7) for the sign TF for the grader to move into the field for the survey. A total of 21 graders passed the exam and participated in these surveys. Training methods in 2015 were the same as those used for the 2012/2013 impact surveys.

Data collection
Household representatives were surveyed about water, sanitation and hygiene (WASH). This included the type of water source, whereby an improved water source was defined according to the WHO’s Joint Monitoring Program definition as a water source that is water piped into a dwelling, water piped to a yard/plot, a public tap or standpipe, a tube well or borehole, a protected dug well, a protected spring or rainwater.14 The availability of water was defined as a time of <30, 30–60 or >60 min to access water and return home. The presence of a latrine was determined by direct observation by the survey teams. All present, consenting individuals within each household were assessed for the WHO simplified signs of trachoma.15 Trachoma graders also made a facial cleanliness determination among children 1–9 y of age at the time of the examination, defined as the absence of nasal and/or ocular discharge.

A random sample of children 1–5 y of age was selected to provide a conjunctival swab to determine the district prevalence of Chlamydia trachomatis infection. Before the start of field work, half of the survey clusters were randomly chosen for swabbing and conjunctival swabs were collected from 25 children per cluster, randomly selected by the data collection software. After trachoma grading, the swab was passed over the conjunctiva three times, rotating 120 degrees between passes. Swabs were placed, dry, into 2-ml vials, which were then placed into coolers with ice packs. Samples were stored in the laboratory at −20°C until they were assayed.

Laboratory assays
At the Amhara Public Health Institute in Bahir Dar, Ethiopia, swab samples for each district were randomized, pooled as five groups of five and processed with the RealTime polymerase chain reaction (PCR) assay (Abbott Molecular, Des Plaines, IL, USA) to detect C. trachomatis DNA using the automated m2000 System (Abbott Molecular). Laboratory technicians were blinded to the district of the sample as well as the trachoma outcomes of the individuals providing the swabs. Strict laboratory quality control procedures were maintained throughout the project. Approximately 10% of the processed pools were exported to external laboratories in the USA each year for repeat testing to validate the results of the RealTime assay in this setting.16

Data analysis
Prevalence estimates were weighted according to the inverse of the probability of selection and robust confidence intervals were calculated taking into account clustering at the village and household levels using Taylor linearization via the svy procedures in Stata 13.1 (StataCorp, College Station, TX, USA). TT was defined according to the WHO guidelines as TT unknown to the health system if individuals ≥15 y of age with TT did not report prior surgery or having been offered and refused surgery in the past.2 Post-stratification weighting, using 5-y age–sex bands from the survey enumerated population, was used when estimating TT prevalence. Prevalence estimates between survey rounds were compared using an unpoled two-sample z-test for proportions to test the differences in prevalence estimates between years. This method accounts for the survey design in the standard errors and avoids multiplicity in the weights due to combining subsequent waves. District C. trachomatis 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.17,18

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

Results
During the 2015 surveillance surveys, 20 849 individuals were enumerated in 5268 households across the five districts. Per district, a median of 1105 households were surveyed and a median of 1031 children 1–9 y of age were examined for the signs of trachoma (Table 1). Among all ages, 14 615/20 849 individuals (70.1%) were present and examined for signs of trachoma, and among children 1–9 y of age, 5384/5635 (95.6%) were present and examined.

In 2015, the prevalence of TF among children 1–9 y of age in all five districts remained below the 5% TF threshold, with a range from 1.5% (95% confidence interval [CI] 0.4 to 4.9) in Injibara to 4.8% (95% CI 2.4 to 9.6) in Finot Selam (Figure 2). The great majority of TF cases were found among children 1–9 y of age, with a peak at ages 3 and 4 y (Figure 3). The TF prevalence among participants ≥10 y of age was 0.2% (95% CI 0.0 to 0.4). Among children 1–9 y of age, the TF prevalence in the 2012/2013 impact surveys were not statistically significantly different than the prevalence in the 2015 surveillance surveys for any district.

In all five surveillance districts, the trachomatous inflammation intense (TI) prevalence among children 1–9 y of age remained low, from 0.7% (95% CI 0.1 to 3.4) in Injibara to 1.2% (95% CI 0.6 to 2.4) in Gende Wuha and 1.2% (95% CI 0.5 to
Table 1. Sample size among the five surveillance districts, Amhara, Ethiopia, 2015

| Zone            | District       | Years since last MDA | Total population | Number of clusters | Number of households | Number of individuals, all ages | Number of children 1–9 y of age |
|-----------------|----------------|----------------------|------------------|--------------------|----------------------|--------------------------------|-------------------------------|
| Awi             | Injibara       | 2.5                  | 25,421           | 16                 | 1,105                | 3,001                          | 1,019                         |
| North Gondar    | Chilga         | 3.5                  | 215,965          | 16                 | 9,600                | 29,484                         | 11,900                        |
| North Gondar    | Genda Wuha     | 2.5                  | 22,473           | 16                 | 11,550               | 28,533                         | 10,311                        |
| North Gondar    | Tach Armachiho | 2.5                  | 106,549          | 16                 | 8,980                | 28,822                         | 11,464                        |
| West Gojjam     | Finot Selam    | 3.5                  | 25,201           | 16                 | 11,500               | 29,311                         | 9,980                         |

Figure 2. Prevalence of TF and TI among children 1–9 y of age at the impact survey (2012/2013) and surveillance survey (2015), Amhara, Ethiopia. TIS: trachoma impact survey; TSS: trachoma surveillance survey.

3.0) in Tach Armachiho. The prevalence of TI remained similar for all districts between the impact and surveillance surveys, except for Injibara, which saw an 85.4% reduction in TI, a difference that was not statistically significant. A small amount of TI was also found among participants ≥10 y of age (79/9218 [1.1%; 95% CI 0.4 to 1.9]) across the five districts.

Approximately 200 children 1–5 y of age were swabbed for ocular *C. trachomatis* in each district. Chlamydial infection was only detected in the district of Finot Selam (1.4%) (Table 2). No infection was detected in Finot Selam in the 2012/2013 impact survey (n=25), nor was it found in any of the other four districts in the 2012/2013 survey. Validation testing of samples assayed in the Amhara Public Health Institute laboratory has demonstrated a high concordance between the lab in Amhara and external reference laboratories. The concordance at the time of this survey was 97%.

After adjusting for age and sex, the prevalence of TT unknown to the health system was 1.7% (95% CI 1.0 to 2.8) in Chilga, 1.2% (95% CI 0.7 to 1.9) in Genda Wuha, 1.0% (95% CI 0.7 to 1.6) in Tach Armachiho, 1.0% (95% CI 0.6 to 1.8) in Injibara, and 2.0% (95% CI 1.4 to 2.8) in Finot Selam. Across the five districts, among participants with TT who responded to the surgical question, the prevalence of previous TT surgery was 15.5% (95% CI 5.6 to 36.1). Among participants with TT who had not yet had surgery, surgical refusal was 4.5% (95% CI 1.2 to 15.4).

In general, WASH indicators, such as the observed presence of a latrine, improved water source, time to water source (round trip), and facial cleanliness among children 1–9 y of age, remained stable between the two survey rounds (Figure 4). There was a statistically significant decrease in the percentage of households with a latrine in Injibara (98.7% vs 80.1%; p<0.0001) and in Finot Selam (87.5% vs 76.3%; p=0.015). Household latrine presence remained <30% in Chilga and Tach Armachiho at both time points. In Chilga, the percentage of children with a clean face was higher in the surveillance survey than in the earlier impact survey (92.4% vs 79.1%; p=0.020).

Discussion

Surveillance surveys have recently become required for programs to monitor whether the gains a trachoma control program has made are sustained after MDA ends. However, as programs scale up and begin to scale down, surveillance surveys will be conducted in districts encompassed by regions where neighbouring endemic districts remain. In the trachoma hyperendemic region of Amhara, a TF prevalence <5% threshold was maintained in the five districts for a period of at least 2 y after ceasing the MDA program. The prevalence of other active trachoma indicators, including TI and *C. trachomatis* infection, were also low. The prevalence of TT unknown to the health care system remained high enough to warrant continued surgical interventions. Comprehensive TT case finding and TT surgical campaigns should continue throughout these districts.

Initial nationwide surveys demonstrated that prior to the SAFE strategy, Amhara was the most trachoma endemic region in Ethiopia. Within Amhara, early district-level surveys conducted in 2001 demonstrated a TF prevalence as high as 90%, with concurrently high levels of TI and *C. trachomatis* infection. In 2006, a zonal-level survey was conducted to provide evidence for the scale-up of the SAFE strategy regionwide. Those surveys demonstrated that the prevalence of trachoma was hyperendemic regionwide (TF among children 1–9 y of age >30%), as well as in the three zones where these five surveillance districts are located. This led to the scale-up of the SAFE strategy, including five annual rounds of MDA. Impact surveys,
designed to assess the success of the SAFE strategy in Amhara, demonstrated that many districts in Amhara still had high levels of TF, TI and chlamydial infection and that more years of the SAFE strategy were needed throughout most of the region.\textsuperscript{5,6,16,20} Owing to the zonal-level nature of the baseline survey, it was difficult to know the exact epidemiology of trachoma in these five individual districts before the start of the SAFE strategy. After 5 y of SAFE, however, these five districts were below the 5% TF threshold and remained below that threshold after stopping MDA for a period of 2–3 y.

Three of the five districts were peri-urban and therefore may have had a different trachoma epidemiology than that of the rural districts of Amhara. Indeed, these three districts generally had a higher prevalence of the common WASH indicators than the rural districts. A statistically significant decrease in the prevalence of latrines was detected in Injibara and Finot Selam, which might suggest deficiencies in initial latrine construction\textsuperscript{21} or a failure to successfully maintain previously built latrines. Maintaining environmental improvements such as latrine accessibility is important for the sustainability of trachoma control as well as for the general health of the population. Despite the decrease in latrine prevalence, and despite being surrounded by districts with high levels of trachoma, Injibara and Finot Selam remained below the recommended 5% TF threshold for a period of 2 and 3.5 y, respectively. Accordingly, these districts continue to not warrant antibiotic intervention, as per the WHO guidelines. Finot Selam, however, had a TF prevalence of 4.8%. Other markers of active trachoma in that district, such as TI (0.9%) and \textit{C. trachomatis} infection (1.4%), were detectable, suggesting that in areas with low TF, it is still possible for limited chlamydial infection to occur, a finding similar to that demonstrated in earlier surveillance surveys.\textsuperscript{7–9}

Given that the prevalence of TF was close to the 5% threshold in Finot Selam, that the prevalence of latrines appeared to be decreasing and that it is surrounded by a district highly prevalent for trachoma, further surveillance may be warranted to ensure that trachoma control has been sustained.

Two of the five surveillance districts were large rural districts that had a TF prevalence below the elimination threshold after 5 y of the SAFE strategy and remained low in the absence of MDA. As with the three peri-urban districts, it was not possible to determine what the district prevalence of TF was before the start of the SAFE strategy and MDA program. However, the prevalence of TT found in these two districts was similar to that in the other three districts and suggests that, historically, trachoma was endemic. These districts had a low prevalence of observed latrines (<30%) at both time points and had the lowest water accessibility of the five districts surveyed. The reasons for the poor uptake of latrines and improved water sources should be explored further in these districts, as the lack thereof can have negative repercussions on health generally. Conversely, these two districts had a high prevalence of observed facial cleanliness among children 1–9 y of age, with an increase in facial cleanliness observed in Chilga between the impact and surveillance survey. Although it is possible that this increase in facial cleanliness was due to sampling variability between surveys, it could reflect health messaging reaching children through school-based programs in Amhara and could also signify a prioritization of hygiene even under suboptimal water and sanitation conditions. Likely there were unmeasured environmental, WASH and cultural variables that contributed to the lower levels of TF observed in this part of Amhara. Although

![Figure 3. Age distribution of TF and TI among children 1–9 y of age from the surveillance surveys in five districts with TF <5%, Amhara, Ethiopia, 2015.](image)

### Table 2. \textit{Chlamydia trachomatis} infection prevalence in children 1–5 y of age at the impact survey (2012/2013) and surveillance survey (2015), Amhara, Ethiopia

| Zone          | District          | Impact survey | Surveillance survey |
|---------------|------------------|---------------|---------------------|
|               |                  | Number of swabs\textsuperscript{a} | \textit{C. trachomatis} prevalence, % | Number of swabs | \textit{C. trachomatis} prevalence, % |
| Awi           | Injibara         | 21            | 0.0                 | 199             | 0.0             |
| North Gonder  | Chilga           | 197           | 0.0                 | 207             | 0.0             |
| North Gonder  | Gende Wuha       | 20            | 0.0                 | 211             | 0.0             |
| North Gonder  | Tach Armachiho   | 89            | 0.0                 | 211             | 0.0             |
| West Gojjam   | Finot Selam      | 25            | 0.0                 | 211             | 1.4             |

\textsuperscript{a}Sample size determinations for the 2012/2013 impact surveys were based on relative size of each district throughout Amhara.
these districts appear to be no longer at risk for active trachoma infection, a continued focus on environmental improvement is important to improve health outcomes in these districts.

The prevalence of TT unknown to the health system at the time of the surveillance survey demonstrated that increased efforts will be needed in these districts before they can be considered to have reached the elimination threshold as a public health problem. With these districts no longer needing MDA, there may be an opportunity to reallocate resources for increasing surgical output. To date, surgery for TT is not widely available within the health system. Therefore the trachoma control program in Amhara should continue to use camp-based surgical campaigns to reach both urban and rural populations, as well as continue to bolster local capacity for TT case detection and surgical services. Recurrence of TT within resource-poor areas will continue to be a problem facing the global trachoma community and warrants greater focus.72

Based on these experiences in Amhara, national trachoma control programs scaling up the SAFE strategy should expect heterogeneous results, whereby districts reaching the elimination as a public health threshold may be adjacent to districts with a high burden of trachoma remaining. Longitudinal trachoma studies have demonstrated that chlamydial infection can return from low levels once antibiotic treatment is ceased.23,24

Those study populations were surrounded by areas that were not yet receiving MDA, thus population mixing could have led to the observed re-emergence of trachoma. Other studies, however, have demonstrated that although in-migration of individuals from endemic areas may reintroduce infection,25–27 these infections may not necessarily lead to the re-emergence of trachoma transmission.29 These five surveillance districts in Amhara were surrounded by trachoma-endemic districts, however, no considerable increases in TF or chlamydial infection were observed over a period of up to 3.5 y within the surveillance districts themselves. It is likely that since the surrounding districts were using the SAFE strategy, including antibiotic distribution, infection was sufficiently low to avoid cross-border transmission. Whether or not this same level of protection is possible under conditions of suboptimal antibiotic coverage, or after breaks in coverage or in settings with low levels of quality water and sanitation is something for programs to monitor closely as a potential risk to sustainable control.

Because the baseline surveys for these districts were conducted in a larger enumeration unit, namely the administrative zone, it was not possible to estimate what the baseline prevalence of trachoma indicators was prior to the start of the SAFE strategy. This makes it difficult to know whether the impact survey data represented a decrease in prevalence, whether that decrease was as a result of the SAFE strategy or whether the prevalence in these districts was low historically.28 This will be a problem faced by many trachoma programs, as enumeration units larger than the true administration unit for health care are often used to gather evidence to start the SAFE strategy.29,30 Not enough ocular swab samples were collected to statistically demonstrate the absence of infection within these districts; however, the corresponding levels of TI suggest that if infection was present, it was probably present at very low levels.16 Although these surveys exceeded the desired sample size for all five districts, it is possible that trachoma ‘hot spots’ may have been missed. This was likely less of a concern in the three smaller peri-urban districts where a considerable proportion of all available clusters were surveyed. More trachoma-specific research is needed to better understand optimal cluster counts and households per cluster that balance cost efficiency and precision,32 such as being done with schistosomiasis,33 and strategies for longer-term surveillance in former trachoma-endemic districts need further development.3

In a hyperendemic setting for trachoma, five districts were below the 5% elimination threshold for TF as a public health problem and remained low after up to 3.5 years without MDA. The elimination threshold for TT has not yet been reached in any of these districts and improvements in water and sanitation coverage are needed. Therefore the surgery, facial cleanliness and environmental improvement components of the SAFE strategy should continue.

**Authors’ contributions:** SDN, AEPS, TA, ES, MZ, MC, ZT and EKC designed the study. TA, ES, MZ, DG, BM, GA, ZA and BB performed the study fieldwork. SDN, AEPS, TA, ES, ZT and EKC analysed the data and wrote the first draft of the manuscript. SDN, AEPS, TA, ES, MZ, DG, BM, GA, ZA, BB, MC, ZT and EKC revised the manuscript. All authors read and approved the final manuscript. SDN is the guarantor of the paper.

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