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Surveillance Surveys for Reemergent Trachoma in Formerly Endemic Districts in Nepal From 2 to 10 Years After Mass Drug Administration Cessation

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IMPORTANCE To verify districts for elimination of blinding trachoma, the World Health Organization requires a population-based surveillance survey for follicular trachoma (TF) and trachomatous trichiasis (TT) 2 years after mass drug administration (MDA) activities have ceased. However, it is unknown if 2 years provides enough time to discover reemergence.

OBJECTIVE To determine the prevalence of trachoma from surveys among 4 districts in Nepal (Dailekh, Dang, Surkhet, and Kanchanpur) that had surveillance intervals of 2, 4, 8, and 10 years, respectively, after cessation of MDA.

DESIGN, SETTING, AND PARTICIPANTS Cross-sectional surveys were carried out in 2015 and 2016. Data analyses were done from March to September 2016. Among 20 clusters randomly selected from each district, 15 were randomly selected for infection and antibody testing: TF and TT were assessed, conjunctival swabs were tested for chlamydial infection, and blood spots were collected on filter paper to test for antibodies to *Chlamydia trachomatis* pgp3 using a multiplex bead assay. The study setting was 4 districts previously endemic for trachoma in Nepal. Participants were randomly selected and included 50 children aged 1 to 9 years and 100 adolescents and adults 15 years and older from each of the 20 clusters; this investigation reports on the children.

MAIN OUTCOMES AND MEASURES Length of time since the last round of MDA and the prevalence of TF among children aged 1 to 9 years and the prevalence of TT among adolescents and adults 15 years and older.

RESULTS Of 3024 children surveyed in the clusters, 48.0% (n = 1452) were female. The mean (SD) age of the children was 5.4 (2.6) years. Eleven cases of TF were found, with a TF prevalence less than 1% in all 4 districts. Three cases of infection were found. Seropositivity for pgp3 antibody varied from 1.4% (95% CI, 0.7-2.6) in the district with a 10-year surveillance interval to 2.5% (95% CI, 1.3-4.5) in the district with a 4-year surveillance interval. Seropositivity increased slightly with age in only one district. The TT prevalence was less than 1 case per 1000 among the total population in all 4 districts after accounting for cases known to the health system and cases with no scarred conjunctiva.

CONCLUSIONS AND RELEVANCE This study found no evidence of reemergence of trachoma up to 10 years after cessation of MDA in 4 districts in children in Nepal. The recommendation for a surveillance survey at 2 years, as proposed by the World Health Organization, is supported by these data. Determining if individuals with TT had scarring or are known to the health system was critical for meeting elimination criteria of blinding trachoma.

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Trachoma, a chronic conjunctivitis caused by *Chlamydia trachomatis*, is the leading infectious cause of blindness worldwide, although impressive strides toward elimination are being achieved.[1] The World Health Organization (WHO)[2] has set a goal of elimination of trachoma as a public health problem by 2020. Elimination has been defined as a follicular trachoma (TF) prevalence less than 5% among children aged 1 to 9 years at the district level and as a trachomatous trichiasis (TT) case unknown to the health system prevalence less than 2 cases per 1000 population 15 years and older at the district level.[3] As more endemic districts within countries reach the elimination goal, they must undergo surveillance surveys for potential reemergence. The WHO recommends that at least 2 years after cessation of mass drug administration (MDA) a surveillance prevalence survey should be undertaken in the district to reconfirm a less than 5% level of TF and a sustainable reduction of TT.[3]

However, the appropriate interval is unknown between cessation of MDA and resurvey to detect reemergence of TF. The expert opinion[3] that suggested the 2-year period was based on balancing the need to detect reemergence early enough to enable prompt management and the need to provide sufficient passage of time for reemergence to occur. Based on scant research in which MDA was stopped and infection reemerged, it appeared reasonable to propose a 2-year interval.[4-6] Nonetheless, data are needed to support the use of a 2-year interval and to investigate if an assessment of infection or alternative tools provide additional information with clinical assessment of TF on potential reemergence. A surveillance survey was conducted 4 years after MDA cessation in a district in Tanzania, which found no evidence of reemergence of TF and a low but modest age-specific increase in levels of antibodies to pgp3 antigen.[7]

Nepal is undergoing surveillance surveys for its 20 formerly endemic districts that have stopped MDA at various times in the past after undergoing trachoma impact surveys indicating that TF was below WHO thresholds for intervention with antibiotics. We have previously reported data from 2 districts in Nepal that had residual TF at the time of impact survey and that had stopped MDA 2 and 4 years, respectively, before the surveys.[8] There was no evidence of reemergence in those 2 districts. Among the 20 districts in Nepal, we have now investigated reemergence in 4 districts (Dailekh, Dang, Surkhet, and Kanchanpur) that had stopped MDA programs at 2, 4, 8, and 10 years, respectively, before the planned surveillance surveys. We nested an assessment of infection with *C trachomatis* and a test for antibodies to *C trachomatis* pgp3 antigen in a random sample of clusters for the surveillance surveys for these 4 districts. We hypothesized that there would be no difference in the TF prevalence according to the number of years since the last MDA in these districts.

Key Points

Question Is the World Health Organization recommendation for surveillance surveys to be done 2 years after cessation of mass drug administration in districts formerly endemic for trachoma sufficient to detect reemergence of disease?

Findings Cross-sectional surveys of 3024 children aged 1 to 9 years were done in 4 districts that were 2, 4, 8, and 10 years, respectively, after mass drug administration. No evidence of reemergence of trachoma or trichiasis exceeding 1 case per 1000 population was identified.

Meaning These data support the World Health Organization recommendation of surveillance surveys at 2 years after the last mass drug administration.

Methods

Ethical Approval

This study was approved by the Johns Hopkins Institutional Review Board, the Nepal Netra Jyoti Sangh, and the Nepal Health Research Council. Written informed consent was obtained from the guardians of each child, and all adolescents and adults provided written informed consent for their participation.

Population

Nepal is divided into 75 districts, of which 20 are formerly endemic for trachoma. We chose 4 districts because they had a TF prevalence less than 5% at their most recent impact survey and were 2, 4, 8, and 10 years, respectively, since their last MDA. We refer to this period as the surveillance interval. Each district had 3 rounds of MDA with azithromycin before MDA activities ceased. Programs on improving the environment and promoting facial hygiene continued, as did the community screening for individuals with TT, who were referred to their local eye hospital for surgery.

The districts contain villages, called Village Development Committees (VDCs), which are the smallest administrative unit and can vary from 300 persons to 35 000 persons. The VDCs are further divided into wards, of which there are at least 9 per VDC.

Selection of Clusters

The 2011-2012 Nepal National Census provided a complete list of wards within the 4 districts and their population size. To make clusters of equal size, we geographically divided wards with a population exceeding 300 to create several clusters in such a way that each cluster contained between 150 and 300 people. Wards with populations between 150 and 300 were kept intact, and wards with populations less than 150 were combined to obtain the target population. A total population between 150 and 300 is expected to yield at least 50 children between the ages of 1 and 9 years per cluster, which was specified for the survey. Twenty clusters were randomly selected from each district for the surveillance survey, and the first 15 of these 20 randomly selected were assigned to the ancillary study of infection and antibodies.

A community volunteer went house to house after a random start and registered up to 50 children aged 1 to 9 years and 100 adolescents and adults 15 years and older for the survey. All children of eligible age and all adults in the house were

invited to participate, so in each cluster more than 150 persons may have been invited. In total, across the 4 districts, we have clinical trachoma assessments on 4042 children and 8103 adolescents and adults. This investigation subsequently reports on the children. In each district, infection data were available for 50 children aged 1 to 9 years from each of the 15 clusters (3024 children). In the first 2 districts, only children aged 1 to 4 years and aged 9 years from the 15 clusters were included in the study on antibodies owing to funding restrictions. However, in the last 2 districts, all children in the sample from the 15 clusters were included in the study on antibodies.

Survey

Cross-sectional surveys were administered in 2015 and 2016. Data analyses were done from March to September 2016. The survey involved 3 components, including grading for TF and TT (and trachomatous scarring where TT was found), in all 20 clusters and districts. Among children in the clusters, a conjunctival swab of the right eye was obtained for testing of *C trachomatis*, and a finger prick was performed for collection of blood for antibody testing. These components are described in more detail below in the Infection Assessment and Antibody Assessment subsections.

Clinical Assessment

Before each survey, retraining was conducted with a Global Trachoma Mapping Project–certified grader who used the presentation slides from the certification course as a refresher. All graders had to pass the teaching slides component with a κ statistic exceeding 0.7 to participate in the survey. For 3 of the districts, a Global Trachoma Mapping Project–certified grader administered the survey. Graders conducted the examination for TF and TT using the WHO simplified grading scheme,[9] a flashlight, and a ×2.5 magnification loupe. If TT was found, the eyelid was everted to ascertain if scarring was present. If no scarring was present, the TT was not attributed to trachoma. All individuals with TT were further queried as to whether they had undergone trichiasis surgery, had been offered surgery, or would like the team to refer them. If an individual indicated that he or she had undergone surgery already or had been offered surgery and refused, that person was designated as a TT case known to the health system.

Infection Assessment

For infection assessment, the right upper eyelid was everted, and a dry swab was obtained of the upper conjunctiva. Strict adherence to protocol was observed to avoid field contamination, and 3 control (air) swabs per cluster were obtained in the field to monitor possible contamination. These controls were labeled and analyzed in an identical fashion to true specimens. The swabs were placed in a swab transport reagent tube (Cepheid), kept cold in the field, and processed the next day by a trained technician in the local district hospital using an automated platform (GeneXpert; Cepheid). Results were reported as positive or negative. Known positive and negative chlamydia controls (Zeptometrics) were run weekly to calibrate the machine.

Antibody Assessment

For antibody assessment, blood was collected by finger prick from each eligible child onto filter paper with circular extensions, calibrated to collect 10 μL of whole blood (filter paper disks; TropBio). These specimens were air dried, stored in plastic bags, and shipped to The Johns Hopkins University. The blood spots were analyzed for antibody to chlamydial antigen pgp3 as previously reported[10] using a multiplex bead assay on a diagnostic platform (Luminex 100; Luminex Corporation). Results are reported as the median fluorescence intensity minus background (MFI minus BG). The positivity cutoff was determined by receiver operating characteristic curve analyses as described previously.[10]

Statistical Analysis

The overall district prevalence of antibody, infection, or TF was estimated as the number of positives in the age groups studied over the total sample in the age group studied. The proportion of children who were seropositive for antibodies against pgp3 is presented for each age, noting that ages 5 to 8 years were not done for the first 2 districts. A test for trend (Mantel-Haenszel χ^2_1) was used to investigate increased antibody positivity with age. The magnitude of clustering of antibody positivity within sampled research clusters was assessed using the intraclass correlation coefficient; the point estimate and 95% CI are reported. To calculate the prevalence of new cases of TT per the total population in the district, we calculated the age-specific prevalence among our sample using the age groups 15 to 39 years (where there were no cases), 40 to 49 years, 50 to 59 years, 60 to 69 years, and 70 years and older. These age group–specific rates were applied to the population 15 years and older in each district, assuming that there were no cases in the population younger than 15 years. This number of expected cases divided by the total population of the district provides an estimate of the age-adjusted prevalence of new TT cases per the total population of the district. The rate and 95% Poisson CI are presented for the prevalence of TT. All *P* values are 2-sided.

Results

Of the 3024 children surveyed in the clusters, 48.0% (n = 1452) were female. The mean (SD) age of the children was 5.4 (2.6) years.

There was no evidence of reemergence of trachoma by surveillance interval (**Table 1**). All districts had a TF prevalence less than 1%. Only 3 positive tests for infection were found in the entire study, 1 in the district that had a surveillance interval of 2 years and 2 in the district that had a surveillance interval of 8 years.

Seropositivity for antibodies to pgp3 was low and showed no statistically significant age-specific increase in 3 of the 4 districts (**Table 2**). The highest rates were among the 9-year-olds and were 4.3% in the districts that had surveillance intervals of 4 and 8 years. The overall rates of seropositivity for pgp3 antibody varied from 1.4% in the district with a 10-year surveillance interval to 2.5% in the district with a

Table 1. Trachoma Prevalence and Infection Prevalence Among Children Aged 1 to 9 Years in 4 Districts in Nepal According to the Surveillance Interval (Time From Impact Survey to Surveillance Survey)

District	Surveillance Interval, y	Surveillance Survey Results	
		Trachoma Prevalence, No./ Total No. (%) [95% CI][a]	Infection Prevalence, No./ Total No. (%) [95% CI][b]
Dailekh	2	1/1009 (0.1)[0.0-0.6]	1/759 (0.1)[0.0-0.7]
Dang	4	1/1012 (0.1)[0.0-0.6]	0/761 (0.0)[0.0-0.5]
Surkhet	8	1/1011 (0.1)[0.0-0.6]	2/752 (0.3)[0.0-0.9]
Kanchanpur	10	8/1010 (0.8)[0.3-1.6]	0/752 (0.0)[0.0-0.5]

[a] Estimated from all 20 clusters per district.

[b] Estimated from the 15 clusters per district.

Table 2. Percentage of Seropositivity for Antibodies to pgp3 by Age in the Districts

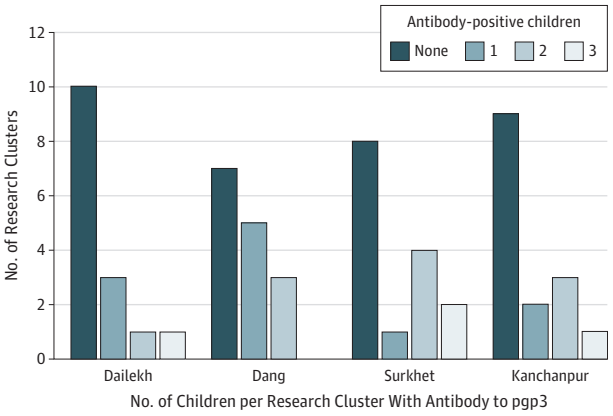
Age, y	No./Total No. (% Positive)			
	Dailekh[a]	Dang[a]	Surkhet	Kanchanpur
1	0/51 (0)	1/94 (1.1)	0/56 (0)	1/39 (2.6)
2	2/56 (3.6)	2/81 (2.5)	1/71 (1.4)	1/94 (1.1)
3	1/68 (1.5)	4/90 (4.4)	1/71 (1.4)	3/104 (2.9)
4	2/70 (2.9)	0/79 (0)	1/65 (1.5)	0/77 (0)
5	NA	NA	1/97 (1.0)	1/86 (1.2)
6	NA	NA	1/78 (1.3)	1/73 (1.4)
7	NA	NA	0/80 (0)	2/66 (3.0)
8	NA	NA	4/96 (4.2)	0/95 (0)
9	3/111 (2.7)	4/94 (4.3)	6/138 (4.3)	2/121 (1.7)
Age range, y				
1-4	5/245 (2.0)	7/344 (2.0)	3/263 (1.1)[b]	5/314 (1.6)
5-8	NA	NA	6/351 (1.7)[b]	4/320 (1.3)
9	3/111 (2.7)	4/94 (4.3)	6/138 (4.3)[b]	2/121 (1.7)
Overall	8/349 (2.3) [95% CI, 1.0-4.4]	11/438 (2.5) [95% CI, 1.3-4.5]	15/752 (2.0) [95% CI, 1.1-3.3]	11/775 (1.4) [95% CI, 0.7-2.6]

Abbreviation: NA, not applicable.

[a] Children aged 5 to 8 years were not tested in these 2 districts.

[b] $P = .04$ by test for trend with age.

Figure. Number of Clusters Within 4 Districts in Nepal That Had 0, 1, 2, or 3 Children With Seropositivity for Antibodies to pgp3



The intraclass correlation coefficient was 0.155 ($P = .21$).

4-year surveillance interval. There was no evidence of clustering of antibody positivity (**Figure**). Only 4 clusters had 3 children who were seropositive, and these cases were spread over 3 districts.

The total number of cases of trichiasis found in all 4 districts was 38. Of these, 3 individuals had no scarring and were not considered TT cases, and 26 individuals had undergone previous trichiasis surgery or had been offered surgery and refused and were not considered new cases. Nine TT cases were new to the health system. As summarized in **Table 3**, no district had a prevalence of 1 case per 1000 total population or higher. The greatest number of trichiasis cases was in the district that had the longest surveillance interval, and all but 3 cases were already known to the health system.

Discussion

The assessment of the prevalence of clinical trachoma found no difference across 4 districts in Nepal, with no evidence of reemergence up to 10 years after cessation of MDA in children, suggesting that 2 years was a reasonable interval after which to conduct the surveillance survey. The test of infection provided no additional data that were not already known from the clinical assessment. The availability of the Cepheid GeneXpert machine in the field was helpful in determining infection data in virtually real time but in the end did not add information to the conclusion about the absence of reemergence.

The levels of seropositivity to pgp3 were among the lowest reported to date. In another surveillance survey in Tanzania, where the rate of trachoma was low at 0.4%, seropositivity ranged from 5% in individuals aged 1 to 4 years up to 9% in individuals aged 7 to 9 years, with a small but statistically significant increase with age.[7] An impact survey in another district in Nepal also found a low rate of trachoma at 0.3%; in 3

Table 3. Trachomatous Trichiasis (TT) Prevalence by District

Variable	Dailekh	Dang	Surkhet	Kanchanpur
Cases of TT, No.	1	8	6	22
Cases of TT with no scars, already operated, or refused, No.	1	6	2	19
Cases of TT new to the health system, No.	0	2	4	3
Age-specific TT prevalence, No./total No. (%)				
15-39 y	0/1210 (0.0)	0/1125 (0.0)	0/1088 (0.0)	0/1094 (0.0)
40-49 y	0/286 (0.0)	0/288 (0.0)	2/267 (0.75)	0/298 (0.0)
50-59 y	0/213 (0.0)	0/260 (0.0)	1/270 (0.37)	2/247 (0.81)
60-69 y	0/198 (0.0)	0/238 (0.0)	0/235 (0.0)	0/239 (0.0)
≥70 y	0/106 (0.0)	2/122 (1.6)	1/167 (0.60)	1/152 (0.66)
Expected cases of TT in the district, No.	0	197	345	293
District-level age-adjusted TT prevalence per 1000 population, % (95% CI)	0.0/1000 (0.0/1000-0.01/1000) population	0.36/1000 (0.31/1000-0.41/1000) population	0.98/1000 (0.88/1000-1.09/1000) population	0.65/1000 (0.58/1000-0.73/1000) population

communities where 68 children were tested, one child was found to be seropositive, with very wide 95% CIs that include our estimate of 1.9%.[11] In another subvillage in Tanzania that had no known infection since 2005, a survey in 2014 found 3.5% seropositivity in individuals aged 1 to 9 years; data were not published any finer than the overall category.[12] In a study[13] reporting data from individuals aged 1 to 9 years in Laos and Uganda, both sites had low trachoma prevalence; the overall TF rate was 1.6% in Laos among 3 provinces, and the overall rate of TF was 3.4% in Uganda. Using an enzyme-linked immunosorbent assay test for antibodies and visual inspection of the inflection point to determine seropositivity, the authors estimated surprisingly high seroprevalences of 10% in Laos and 24% in Uganda. Using the same external standards as we used, seropositivity rates were 6.7% in Laos and 6.8% in Uganda, which are more similar to previous rates found in low endemic areas.

Our data suggest that both the low rate of seropositivity and an absence of a marked age-specific increase in seropositivity were hallmarks of the lack of reemergence. This finding was particularly notable because the lowest rate for the individuals aged 9 years among the 4 districts in Nepal was in the group with a 10-year interval between stopping MDA and the surveillance survey. The similarity in rates of seropositivity for those aged 9 years compared with those aged 1 to 4 years suggests an absence of transmission over the 10 years since MDA. The individuals aged 9 years in the other districts had seropositivity rates slightly higher than those of the individuals aged 1 to 4 years, as expected because the 9-year-olds were born before the program commenced or during program start-up.

The rate of TT unknown to the health system was less than 1 case per 1000 population in each of the districts. The algorithm for determining this rate worked well and revealed 3 individuals with trichiasis who did not have a conjunctiva with scars visible under ×2.5 magnification. Scarring is the mechanism that leads to trichiasis due to trachoma; if none is visible, the trichiasis could be due to other causes and should not be formally considered TT. Technically, one could posit invisible scarring, but we argue there is no evidence that nonvisible scarring is truly the type of scarring that leads to blinding trachoma. While individuals who have trichiasis with no scarring can be referred for appropriate eye care follow-up, we believe that these should not be considered TT cases in surveillance surveys.

The largest number of exclusions was due to cases that were already known to the health system either because the individuals had undergone previous trichiasis surgery or were referred, refused, and continued to refuse. Nepal has a good system of female health volunteers who screen for trichiasis in their communities, and they assist the survey team in determining new cases or individuals who have been referred previously. In Kanchanpur, which had 10 years of experience with screening, referrals, and refusers since the last MDA activity, 19 of the 22 cases of TT found via the survey were known to the health system already. This result shows the value of a health care system that continues to identify individuals with trichiasis and offer surgery even as long as 10 years after the MDA component has stopped. There are still new cases in that district, suggesting that ongoing efforts should persist.

Limitations

We acknowledge limitations to this study, which include the obvious small sample size for detecting TT rates as low as 1 case per 1000 population with precision at the district level. The trachoma surveys are powered to detect TF rates in children as low as 4%, with a 95% CI ±2%, and adolescents and adults are screened for trichiasis within this framework.[14] Often, the sample size is approximately 2000 adolescents and adults per district, so just a few cases of TT can make the difference between meeting the TT target or failing. There are efforts to design a TT-only survey, which entails a larger sample size that will provide more stable estimates. Another limitation to our study is the absence of data on antibodies at the time of impact survey. Such data would have enabled us to clarify change over the course of the surveillance period. For example, if individuals aged 9 years at the time of impact survey had higher rates of seropositivity that were lower among the individuals aged 9 years after 8 to 10 years, the inference of interruption of transmission would be much stronger. The use of the younger age groups that were born

after MDA cessation provides some reassurance of low to no transmission. Finally, while all of the districts had residual low levels of trachoma at the time of impact survey, this finding was based solely on clinical assessment, and the reliability is unknown. Although Nepal has historically had a trachoma problem as documented in blindness surveys, reports also exist of secular trends of declining rates in the absence of trachoma control programs.[15] There is the possibility of immigration of persons who had less disease, but this theory is unlikely given that the districts are surrounded by other districts that also had few trachoma cases. Immigration from India is also an issue for districts that are close to the Indian border, but these individuals are more likely to be adolescents and adults seeking TT surgery according to the Nepal trachoma program. Our data suggest that 2 years after impact survey is a reasonable time to conduct a surveillance survey because there is no evidence of reemergence of trachoma in later years, but further data from other countries would be useful.

Conclusions

In summary, we found that determining if individuals with TT have scarring or are cases known to the health system was critical for meeting elimination criteria, especially where an active health system has been providing high-quality surgical services. We observed no evidence of reemergence of trachoma in these 4 districts even at a 10-year surveillance interval in children. The recommendation for a surveillance survey at 2 years, as proposed by the WHO, is supported by these data. For Nepal, delaying the surveys for more than 2 years would provide no additional information about elimination of blinding trachoma.

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Study concept and design: West, Zambrano, Sharma, Mishra, Gaydos, Rotondo.
Acquisition, analysis, or interpretation of data: West, Zambrano, Sharma, Mishra, Muñoz, Dize, Crowley, Gaydos.
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