Prevalence of ocular *Chlamydia trachomatis* and active trachoma among children in Merhabete district, Amhara, Ethiopia

Awraris Hailu Bilchut[1,2], Esmael Habtamu[2,3,4], Yeshigeta Gelaw[4], Aemero Abateneh[4], Belay Beyene[5], Ambahun Chernet[5,6], Tariku Wondie[2], Adisu Abebe[7], Huiyu Hu[8], Hadley Burroughs[8], Zhaoxia Zhao[8], Jessica Shantha[8,10], Scott D. Nash[11,12], Benjamin F. Arnold[8,10], Thomas M. Lietman[8,9,10±], Catherine E. Oldenburg[8,9,10±*]

[1]Debre Berhan University, Debre Berhan, Ethiopia
[2]Eyu-Ethiopia, Bahir Dar, Ethiopia
[3]International Centre for Eye Health, London School of Hygiene and Tropical Medicine, London, United Kingdom
[4]College of Medicine and Health Sciences, Bahir Dar University, Bahir Dar, Ethiopia
[5]Amhara Public Health Institute, Bahir Dar, Ethiopia
[6]The Carter Center, Addis Ababa, Ethiopia
[7]Amhara Regional Health Bureau, Bahir Dar, Ethiopia
[8]Francis I Proctor Foundation, University of California, San Francisco, USA
[9]Department of Epidemiology & Biostatistics, University of California, San Francisco, USA
[10]Department of Ophthalmology, University of California, San Francisco, USA
[11]Rollins School of Public Health, Emory University, Atlanta, GA, USA
[12]The Carter Center, Atlanta, GA, USA

[±]Contributed equally

Author for Correspondence:
Catherine E. Oldenburg, ScD MPH
Francis I Proctor Foundation
University of California, San Francisco
490 Illinois St, Floor 2
San Francisco, CA, 94158
Phone: 415-502-8843
Email: catherine.oldenburg@ucsf.edu

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ABSTRACT

There have been large reductions in the burden of trachoma worldwide. However, some districts have had persistently high trachoma prevalence despite many years of intervention. Here, we report the epidemiology of trachoma in Merhabete, Ethiopia, a district in Amhara Region that has been receiving azithromycin mass drug administration (MDA) since 2009. Data arose from the baseline survey of a cluster randomized trial evaluating targeted treatment strategies for trachoma elimination. An enumerative census was conducted in February 2022 to generate lists of children aged 6 months to 9 years in 80 sentinel communities participating in the trial. All children in the sentinel communities who were on the census were examined. Field grades and conjunctival swabs were collected for assessment of active trachoma (based on clinical assessment) and ocular chlamydia (based on PCR to identify *Chlamydia trachomatis*). 5,935 children were examined in 80 communities. Prevalence of trachomatous inflammation-follicular (TF) was 46.6%, trachomatous inflammation-intense (TI) was 17.5%, and ocular chlamydia was 28.0%. The correlation between TF and ocular chlamydia (correlation coefficient 0.54, 95% confidence interval, CI, 0.34 to 0.70) was similar to the correlation between TI and ocular chlamydia (correlation coefficient 0.49, 95% CI 0.30 to 0.65). The prevalence of ocular chlamydia remained high in this district that had received more than 10 rounds of azithromycin MDA. Ocular chlamydia was moderately correlated with both TF and TI. Intensive interventions may be required to eliminate trachoma in settings with persistently high ocular chlamydia prevalence despite many years of intervention.

INTRODUCTION

Azithromycin mass drug administration (MDA) is highly effective at reducing the prevalence of the ocular strains of *Chlamydia trachomatis* that cause trachoma.[1,23] As a result, tremendous progress has been made towards global control of trachoma.[4] However, some areas of sub-Saharan Africa have had persistently high prevalence of trachoma despite many years of azithromycin MDA, many of which are in Ethiopia.[5] As more districts achieve control of trachoma and phase out of meeting criteria for continued azithromycin MDA, understanding the epidemiology of trachoma at scales smaller than a district may help target interventions and identify hot spots for more intensive intervention.[6]

Trachoma programs use the clinical sign trachomatous inflammation—follicular (TF) to monitor indication for and response to azithromycin MDA. The target of azithromycin is ocular chlamydia, and TF is known to decline more slowly following mass azithromycin than ocular chlamydia.[7] Alternative indicators, including trachomatous inflammation—intense (TI), may be useful for trachoma surveillance. At the district level in a study conducted in a high trachoma prevalence setting, TI was shown to correlate more closely with ocular chlamydia than TF during azithromycin MDA.[8] At the community level, a study in Niger demonstrated strong correlation between TF and TI and ocular chlamydia at baseline that weakened with azithromycin MDA in communities with approximately 20% prevalence at baseline.[7]

Several districts in Amhara, Ethiopia have had persistently high prevalence of TF (e.g., TF ≥ 30%) despite many years of intervention.[9] We evaluated the relationship between TF, TI, and ocular chlamydia at the community level in Merhabete district, Amhara, Ethiopia, with the goal of understanding the small-scale epidemiology of trachoma in a district with hyperendemic trachoma.

METHODS

Study Setting. This study was conducted in Merhabete District in the North Shewa Zone of Amhara, Ethiopia. Azithromycin MDA for trachoma began in Merhabete in 2009. The most recent trachoma impact survey conducted in Merhabete (2019) demonstrated the prevalence of both TF and ocular chlamydia infection to be approximately 30%.[10] The most recent azithromycin MDA prior to data collection was in February 2021, approximately 15 months before sample collection began. Data were collected as part of the baseline assessment of the Kebele Elimination of Trachoma for Ocular Health (KETFO) study (ClinicalTrials.gov NCT03335072). This study was conducted in collaboration between the Amhara Regional Health Bureau, Eyu-Ethiopia, Bahir Dar University, the Amhara Public Health Institute, The Carter Center, and the University of California, San Francisco. The study was reviewed and approved by the institutional review boards at the University of California, San Francisco, Emory University, and the London School of Hygiene and Tropical Medicine, the National Ethics Review Committee of Ethiopia, the Amhara Public Health Institute, and the Ethiopian Food and Drug Administration.

Census. A census of all communities in Merhabete was conducted in February 2022. Of 184 communities (known as *gotts*) identified during the census, a random sample of 80 communities were selected as sentinel communities for trachoma monitoring. In sentinel communities, a door-to-door enumerative census was undertaken in which all residents in all communities were enumerated.

Participants. All children aged 6 months to 9 years in all sentinel communities were eligible for examination. In each study community, a community mobilizer informed residents that the study team would be visiting and to bring children under 9 years of age to a central examination point in the community. We obtained written consent from the caregiver of each enrolled participant in accordance with the Declaration of Helsinki.

Outcome assessments. Clinical examination for signs of trachoma was done in the field according to the World Health Organization (WHO) simplified grading system.[11] Examiners in the field were trained and certified by experienced graders following a standardized training and passing an exam with 50 conjunctival photos with a kappa of > 0.7 versus a gold standard grader (an ophthalmologist with expertise in trachoma). The exam included all trachoma signs (e.g., normal, TF, TI, scarring, and corneal opacity). All graders achieved a kappa of ≥ 0.75. Grading for clinical signs of trachoma was done using 2.5x binocular magnifying loupes with penlight illumination. Ocular chlamydia infection was measured using polymerase chain reaction (PCR) on conjunctival swabs collected in the field. Gloved examiners everted the right upper tarsal conjunctiva and swabbed with a Dacron swab (Puritan Medical Products, Guildford, ME, USA). Swabs were placed in tubes without media, and then stored on ice in the field and in a -20°C freezer until being transported to the Amhara Public Health Institute laboratory for long-term storage at -20°C and processing. Samples were eluted, and individual aliquots were combined into pools of 5, stratified by study community.[12] Pools were processed to detect *C. trachomatis* DNA using the Abbot RealTime assay (Abbot Molecular, Des Plaines, IL, USA) on the m2000 platform as in previous studies.[13] Communities from which 100% of pools were positive were re-tested using pools of 3.[12]

Statistical analysis. Community level prevalence of ocular chlamydia was estimated from pooled prevalence using maximum likelihood methods as previously described.[12] The prevalence of TF and TI were calculated as the total number of positive children divided by the total number of children examined in each community. Binomial 95% confidence intervals (CIs) were calculated for each indicator. To assess spatial clustering of TF, TI, and ocular chlamydia at the community level, we calculated the Moran's I statistic. We calculated Spearman rank correlations to assess the degree of correlation between 1) TF and ocular chlamydia, and 2) TI and ocular chlamydia at the community level. All analyses were done in R version 4.4.0 (The R Foundation for Statistical Computing).

RESULTS

A total of 6,010 children aged 6 months to 9 years were registered for examination in 80 communities, of whom 5,935 (99%) had a field grade recorded and 5,901 (98%) had a conjunctival swab for ocular chlamydia assessment collected. Approximately half (51%) of the examined children were female and median age was 6 years (interquartile range 4 to 9 years; **Table 1**).

The median prevalence of TF among study communities overall was 49% (interquartile range, IQR, 34 to 57%) and TI was 16% (95% CI 6% to 25%). The median prevalence of ocular chlamydia among all study communities was 23% (95% CI 11% to 37%). We found no evidence of spatial autocorrelation of TF (Moran's I -0.03), TI (Moran's I -0.03), or ocular chlamydia (Moran's I -0.02) (**Figure 1**). We found evidence of moderate correlation between TF, TI, and ocular chlamydia. The correlation between mean TF and ocular chlamydia was 0.54 (95% CI 0.34 to 0.70; **Figure 2**) and the correlation between mean TI and ocular chlamydia was 0.49 (95% CI 0.30 to 0.65; **Figure 2**).

DISCUSSION

We document persistent hyperendemic trachoma in this district in Amhara that had been receiving azithromycin MDA for more than 10 years at the time of sample collection. Mass distribution of azithromycin has been shown to dramatically decrease the prevalence of ocular

Chlamydia trachomatis and clinical signs of trachoma.[1,2,14,15] While many formerly endemic districts globally have achieved elimination of trachoma as a public health problem, a number of districts have experienced persistently high prevalence of trachoma despite many years of intervention.[16] Ethiopia has the greatest number of these districts, although overall many formerly hyperendemic districts in Ethiopia have now achieved trachoma control.[16] These results, in combination with district-level survey results from Amhara[17], suggest that more intense interventions may be required to achieve trachoma control in districts that continue to have persistently high trachoma prevalence despite many years of intervention.

At the zonal level, previous studies in Amhara have shown that TI may be more closely correlated with ocular chlamydia compared to TF[8], although others at the district level have shown TF to be more closely correlated with ocular chlamydia than TI.[17] In general, the correlation between TF and ocular chlamydia has been higher at the district level in districts with higher TF prevalence.[17] In the present analysis, we found similar correlations between TI and ocular chlamydia and TF and ocular chlamydia. Although the correlation coefficient was slightly higher between TF and ocular chlamydia compared to TI, the 95% confidence intervals overlapped, suggesting there may be minimal true difference in correlation between TF and TI. Despite very high TF prevalence, the correlations observed at the individual community level between both TF and TI and ocular chlamydia were moderate compared to what has previously been shown in district-level analyses.[17]

In Amhara and elsewhere, the prevalence of TI and ocular chlamydia are typically more similar to one another than TF and ocular chlamydia.[7,8] In the present study, ocular chlamydia prevalence was higher than TI prevalence but lower than TF prevalence. Both TF and TI are lagging indicators of ocular chlamydia infection; typically, infection is present prior to development of TF and/or TI and clear before TF and/or TI resolve.[3] Ocular chlamydia has been found more frequently in children with TI compared to TF.[18,19] The present results may suggest high levels of transmission of ocular chlamydia that have not manifested as TI. Monitoring ocular chlamydia infection requires substantial costs and logistics. Alternative indicators, including TI and serology, have been considered to complement the evaluation of TF and better identify districts requiring further intervention for trachoma. These results suggest that TI may underestimate the prevalence of ocular chlamydia at the community level in high prevalence areas, and thus it may not be an adequate alternative indicator for ocular chlamydia if the goal of surveillance is to detect ongoing transmission of ocular chlamydia.

Like other areas receiving azithromycin MDA, Merhabete experienced treatment interruptions during the COVID-19 pandemic.[20] One azithromycin MDA was missed during the height of the COVID-19 pandemic in 2020. More recently, Merhabete and other regions of Amhara have experienced treatment interruptions due to the civil war. In the present study, samples were collected approximately 15 months after the most recent azithromycin MDA. Treatment interruptions or delays may increase ocular chlamydia transmission, contributing to the high prevalence of ocular chlamydia and TF observed in the present study. Given the cross-sectional nature of the current study and lack of data from before pandemic and civil war, we are unable to comment on whether these treatment interruptions have increased transmission of trachoma. However, the prevalence of TF has remained above 30% and infection above 10% despite continuous rounds of intervention. Settings similar to Merhabete will likely need more intensive treatments to achieve control of trachoma as a public health program, and alternative antibiotic distribution strategies should be considered in these contexts.

In this population-based survey using baseline data from a community randomized trial, we demonstrated a very high prevalence of ocular chlamydia and the clinical signs TF and TI in a district in Amhara that had received more than 10 years of MDA with azithromycin. In addition to high prevalence, we found no evidence of spatial correlation and high prevalence of infection was found throughout the district. At the community level, correlations between TF and TI and ocular chlamydia were considerably more moderate than has been observed in previous studies at the district level, and the prevalence of TI was substantially lower than ocular chlamydia. These results suggest that TI alone may not be an adequate alternative indicator for ocular chlamydia infection. Given the high prevalence of TF and ocular chlamydia despite many years of azithromycin distribution, these results suggest that this setting is ideal for evaluating intensive strategies for trachoma control.

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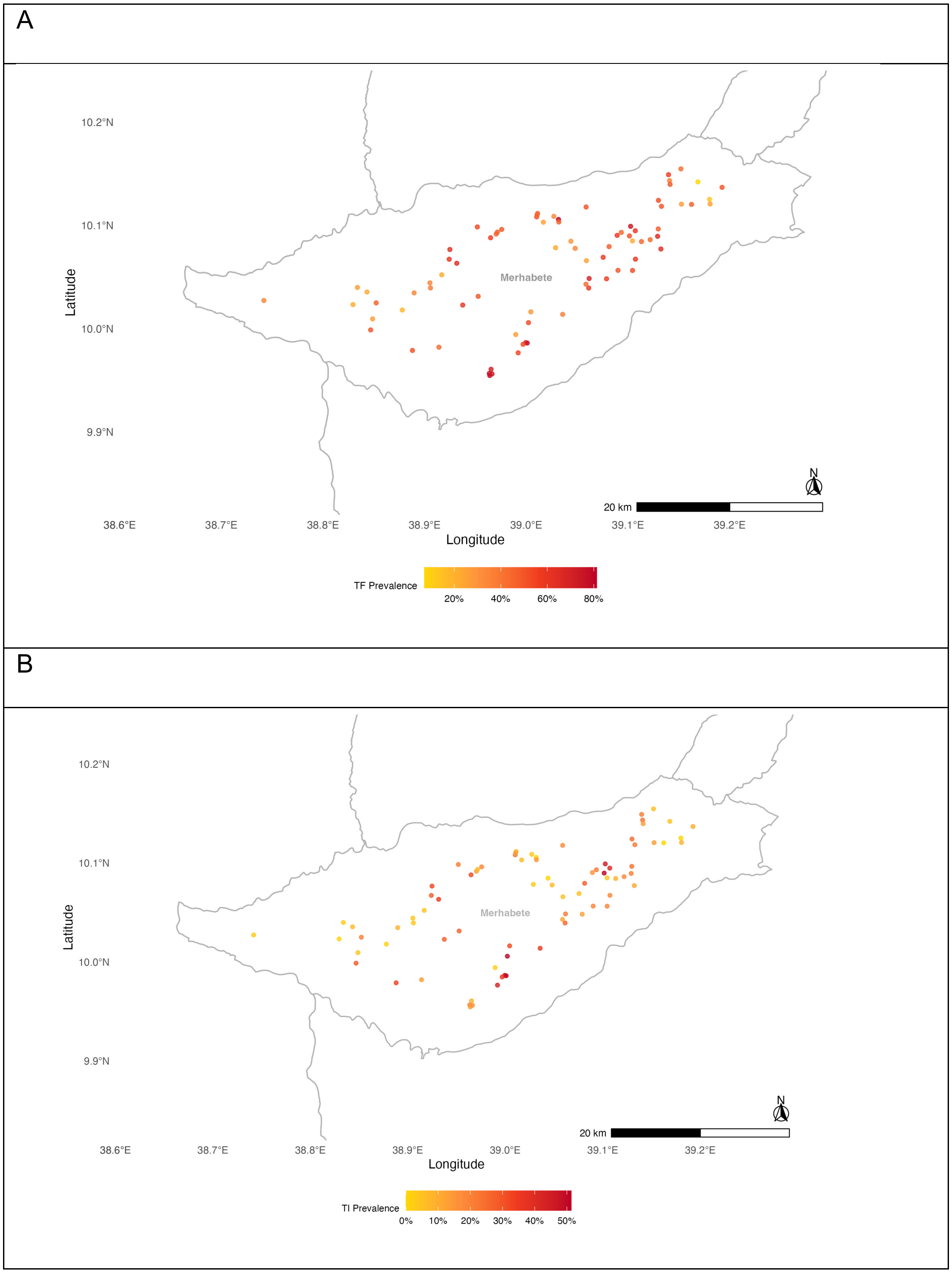
Table 1. Demographic and trachoma characteristics of children surveyed at baseline, KETFO Study, 2022.

	Outcome
Child's sex, N (%)	
Female	3,306 (50.5%)
Male	2,974 (49.5%)
Age, years, median (IQR)	6 (4 to 9)
TF prevalence (95% CI)	46.6% (43 to 50.3%)
TI prevalence (95% CI)	17.5% (14.5 to 20.5%)
Ocular chlamydia prevalence (95% CI)	28.0% (23.1 to 32.9%)

FIGURE LEGENDS

Figure 1. Prevalence of trachomatous inflammation—follicular (A), trachomatous inflammation—intense (B), and ocular chlamydia (C) in Merhabete, Ethiopia at the community level. Darker red colors indicate higher prevalence of each indicator.



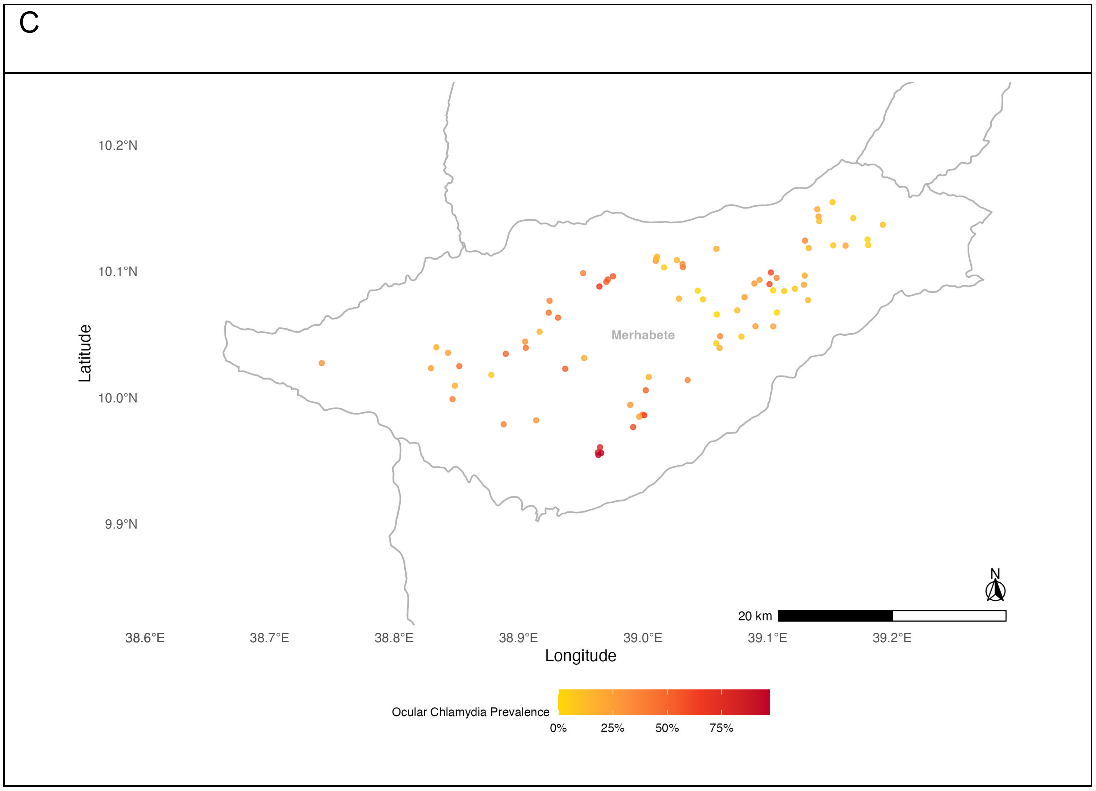
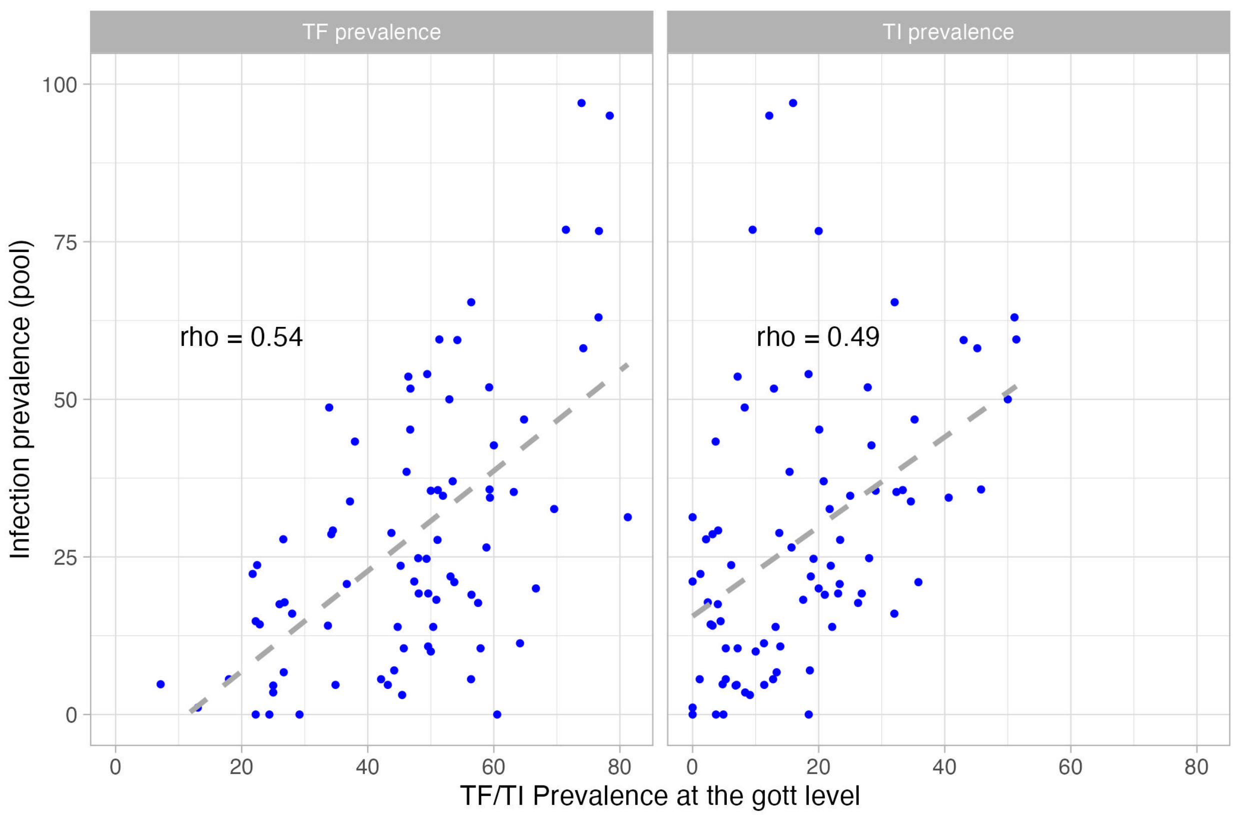

C
10.2°N
10.1°N
10.0°N
9.9°N
Latitude
Merhabete
20 km
N
38.6°E
38.7°E
38.8°E
38.9°E
39.0°E
39.1°E
39.2°E
Longitude
Ocular Chlamydia Prevalence
0%
25%
50%
75%

Figure 2. Correlation between trachomatous inflammation—follicular (TF, left) and trachomatous inflammation—intense (TI, right) and ocular chlamydia prevalence at the community level.



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