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## Seroreversion to *Chlamydia trachomatis* Pgp3 Antigen Among Children in a Hyperendemic Region of Amhara, Ethiopia

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Monitoring trachoma transmission with antibody data requires characterization of decay in IgG to *Chlamydia trachomatis* antigens. In a 3-year longitudinal cohort in a high-transmission setting, we estimated a median IgG half-life of 3 years and a seroreversion rate of 2.5 per 100 person-years (95% confidence interval, 1.6–3.5).

**Clinical Trials Registration.** NCT02754583.

**Keywords.** *Chlamydia trachomatis*; trachoma; antibody; IgG; seroepidemiology; Ethiopia.

Trachoma, caused by repeated conjunctival infection with *Chlamydia trachomatis*, is targeted for elimination as a public health problem by 2030. Control programs currently rely on clinical markers, including trachomatous inflammation-follicular (TF), an early indicator of inflammation, and trachomatous trichiasis, a sign of severe disease progression. However, clinical signs are prone to measurement error. Polymerase chain reaction (PCR)-detected chlamydia infections are an objective alternative, but as populations approach elimination infections become rare, which poses a challenge for surveillance [1]. Antibody responses among children are being explored as an objective, sensitive marker of chlamydia exposure across transmission settings [2].

Population-level monitoring of trachoma using serology has focused on immunoglobulin G (IgG) responses to the Pgp3

antigen [2]. Studies have demonstrated marked increases in seroprevalence with age in trachoma endemic regions and limited antibody responses in populations near elimination [3–5]. A key summary statistic from the age-seroprevalence curve is the seroconversion rate, which is one measure of chlamydia force of infection [6]. In the absence of seroreversion, IgG seropositivity by age reflects the cumulative incidence of prior exposure to chlamydia infection in the population. Seroreversion is a change from seropositive to seronegative status, and, if common, then failing to account for seroreversion could bias estimates and the interpretation of standard analyses of age-seroprevalence curves. Few longitudinal studies have measured Pgp3 IgG decay over time, and all have been conducted in Tanzania's Kongwa District. Seroreversion rate estimates range from 0 cases within 6 months after mass drug administration (MDA) of azithromycin [7] to 12.1% over 36 months (4 per 100 child-years) [8], 6.4% after 1 year (6.4 per 100 child-years) [9], to 4.0% after 6 months (8 per 100 child-years) [10].

Better characterization of seroreversion and the rate at which Pgp3 IgG levels decay among children across a range of transmission settings will aid the development and interpretation of seroepidemiologic models of trachoma transmission. Our goal was to estimate the decay rate of Pgp3 IgG levels and the seroreversion rate in a longitudinal cohort of children 1–9 years old in a hyperendemic region of Amhara, Ethiopia.

### METHODS

The WASH Upgrades for Health in Amhara (WUHA) cluster-randomized trial studied the effect of an integrated water, sanitation, and hygiene (WASH) intervention on ocular chlamydial infection (NCT02754583) [11]. The study enrolled 40 rural communities in the Wag Hemra Zone. Clinical disease, chlamydia infections, and serology were measured annually for 3 years in a longitudinal cohort of children aged 0–5 years at baseline. Approximately 30 randomly selected children per community were included, and infants under 1 year of age were newly enrolled in the longitudinal cohort at each annual visit. Once a child was enrolled in the longitudinal cohort, the study continued to test for infection and antibody responses even if they were 6–9 years old.

Conjunctival swabs were collected from a random sample of 30 children per community at each visit, often including children in the longitudinal cohort. Swabs were tested for chlamydia DNA at the Amhara Public Health Institute in Bahir Dar, Ethiopia using the Abbott RealTime assay [11]. Dried blood spots were collected on TropBio filter paper and tested for IgG responses to Pgp3 in a multiplex bead assay on the Luminex platform using the same bead set for all samples.

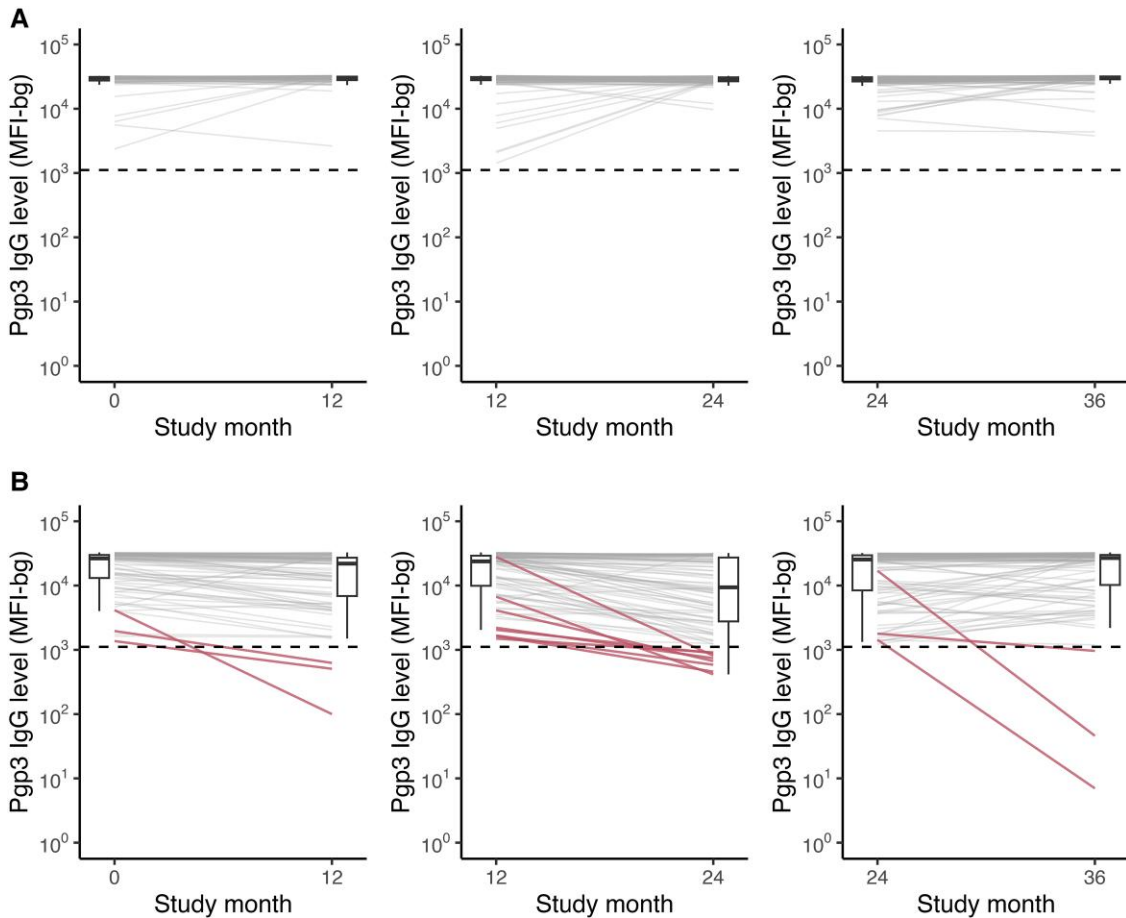
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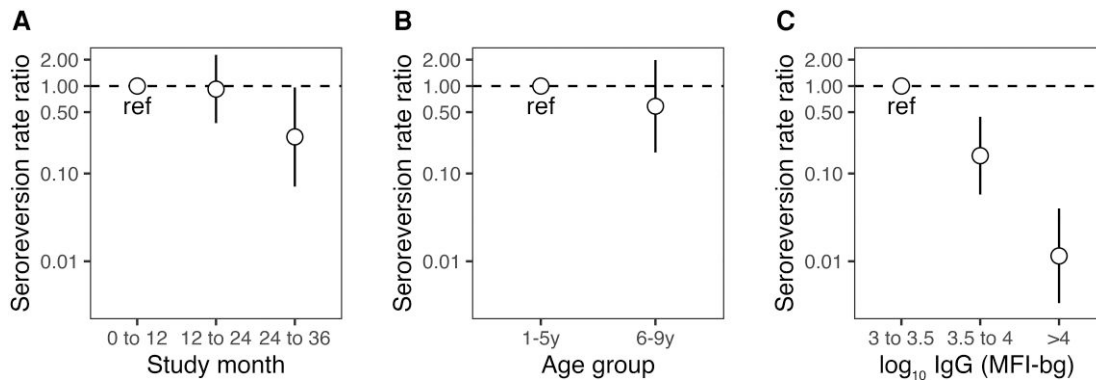
**Figure 1.** Antibody responses over time by *Chlamydia trachomatis* infection status. Pgp3 IgG responses among children who were seropositive at the beginning of each 1-year interval and (A) PCR-positive compared to (B) PCR-negative or equivocal for *C. trachomatis* infection at the end of the interval. Dashed horizontal line represents seropositivity cutoff of 1113 MFI-bg. B, Solid, heavy lines identify children who seroreverted. Boxplots summarize median (thick horizontal line) and interquartile range (thin horizontal lines), and 1.5 times the interquartile range (whiskers) at beginning and end of each interval. PCR results at the end of the interval were missing for 784 (32%) intervals due to random sampling for PCR testing in the cohort. Created using script: <https://osf.io/wsvgr>. Abbreviations: IgG, immunoglobulin G; MFI-bg, median fluorescence intensity minus background; PCR, polymerase chain reaction.

IgG was quantified using median fluorescence intensity minus background (MFI-bg) and responses >1113 were classified as seropositive using a receiver operator characteristic curve cut-off from reference samples [12]. Research was approved by the human subjects review board at the University of California, San Francisco. Each participant or guardian provided verbal consent before any study activity.

Before the trial, MDA was conducted annually for 7 years in the study area but had not sufficiently controlled trachoma [13]. MDA was suspended during the study period. Between baseline and 36 months, TF prevalence among 1–9 year olds remained fairly constant from 63% to 57% [14] and ocular chlamydia infection among 0–5 year olds increased from 11% to 32% with no difference between the control and intervention groups [11].

We estimated incidence rates nonparametrically and using Poisson regression with cluster-level random effects and an offset for person-years. Because this was an open cohort where

children might have incomplete data across time points, we assessed serostatus over 1-year intervals and only included 1-year intervals with serology measurements at both time points. We assumed that changes in serostatus occurred halfway through the year. This analysis focused on the seroreversion rate (SRR), but we additionally estimated the seroconversion rate (SCR) using the same method. Measurements from children <1 year old were excluded from this analysis to avoid the influence of maternal antibody waning. We evaluated differences in SRR by age (1–5 years vs 6–9 years), time period, and initial IgG level by adding covariates to the model. We estimated IgG half-life using an exponential decay model among children who began a 1-year interval seropositive, ended the year with a negative or equivocal PCR test, and did not have an increase in IgG levels. This analysis was conducted in R version 4.3.2 (2023–10–31). Data and replication files are available: <https://osf.io/xquyd/>.



**Figure 2.** Seroreversion by subgroups. *A*, Rate ratios and 95% CIs (on log scale) comparing seroreversion over time. *B*, Rate ratios and 95% CIs (on log scale) comparing seroreversion by age group. *C*, Rate ratios and 95% CIs (on log scale) comparing seroreversion by IgG level at the beginning of the 1-year interval. Created using script: <https://osf.io/wsvgr>. Estimates underlying this figure are provided in [Supplementary Table 3](#). Abbreviations: IgG, immunoglobulin G; CI, confidence interval; MFI-bg, median fluorescence intensity minus background; ref, reference.

## RESULTS

The analysis included 4327 serology measurements from 1511 unique children. We excluded 27 children who were determined to have mislabeled blood specimens at 1 or more time points based on photography, anthropometry, and IgG levels. After filtering to 1-year intervals with serology measurements at both ends, the analysis included 2428 1-year intervals from 1221 unique children (81%). Children who did not contribute were similar to those with partial (47%) or complete (33%) measurements over the 3-year period, but had more missing values for other trachoma indicators ([Supplementary Table 1](#)). Over the study period, seroprevalence among children in the longitudinal cohort increased from 30% to 51% as the cohort aged ([Supplementary Table 2](#)). The SCR in the longitudinal cohort was 15.3 per 100 person-years (95% confidence interval [CI], 11.0–20.8).

Seroreversion was rare: among 886 1-year intervals where children were seropositive at the beginning, 864 (98%) remained seropositive after 1 year. Among seropositive children, IgG levels remained high and were consistent with a durable response ([Figure 1A](#)), although there was some waning of IgG among children who were PCR negative or equivocal for chlamydia infection at the end of the interval ([Figure 1B](#)). There were 22 seroreversions during 875 seropositive person-years at risk, corresponding to an SRR of 2.5 per 100 person-years (95% CI, 1.6–3.5). The estimated median IgG half-life was 3.0 years (interquartile range [IQR], 0.8–9.6). Based on the slope of IgG decay among children who were seropositive at the beginning of the interval and PCR negative/equivocal at the end of the interval ([Figure 1B](#)), there appeared to be 2 decay rates, perhaps due to boosting between measurements or measurement error. Among the 20 children with >4 fold decrease in IgG levels (6 of whom seroreverted), median IgG half-life was 0.38 years (IQR, 0.27–0.44). Seroreversion rates were lower from months 24 to 36 of the study (SRR ratio compared to

months 0 to 12, 0.26; 95% CI, .07–.97; [Figure 2A](#)) and among 6–9 year olds (SRR ratio compared to 1–5 year olds, 0.59; 95% CI, .17–1.98; [Figure 2B](#)), although not statistically significant. Of 22 seroreversions, 14 were among children with IgG MFI-bg <10<sup>3.5</sup> at the beginning of the interval with substantially lower seroreversion rates at higher IgG levels ([Figure 2C](#)).

## DISCUSSION

In a hyperendemic region of Amhara, Ethiopia in the absence of MDA we found that seroreversion was rare and antichlamydia Pgp3 IgG antibody response was durable among children 1–9 years old, with a median half-life of 3 years. The IgG seroreversion rate was 2.5 per 100 person-years (95% CI, 1.6–3.5) and seroreversion rates were lower at the end of the study amidst higher chlamydia transmission, among older children, and among children with higher initial IgG levels. To our knowledge, these are the first estimates of Pgp3 seroreversion rates in a high transmission setting. Strengths of the study include a well-characterized, 3-year longitudinal cohort with paired measures that included clinical signs of trachoma, chlamydia infection measured with PCR, and IgG measured on a multiplex bead assay using a consistent bead set.

Our findings are similar to recent estimates of 6.4% seroreversion after 1 year in the absence of MDA [9] and 4% after 6 months in the presence of MDA in Kongwa, Tanzania [10], but may be slightly lower due to higher ongoing transmission (11% to 32% PCR prevalence) compared to Kongwa (4.9% to 6.3% PCR baseline prevalence). Higher SCR and lower SRR estimates in the present study likely reflect repeated infections that boost and maintain IgG levels above seropositivity cutoffs. Consistent with the results of our subgroup analyses, in Kongwa the SRR decreased with clinical disease activity and higher starting antibody levels.

This analysis had limitations. Children were followed longitudinally but some missed visits, leading to incomplete data. For this reason, we used an open cohort design and focused on changes in serostatus at 1-year intervals to maximize follow-up time for analysis. If children who missed visits were systematically younger with higher seroprevalence, that could lead to a biased underestimate of the SRR. However, children who missed visits appeared similar based on age, seropositivity status, and other trachoma markers at enrollment to those with complete follow-up (Supplementary Table 1). A child could have seroconverted and reverted between annual visits. Although unlikely given the estimated 3-year IgG half-life, if transient changes in serostatus occurred between annual measurements, perhaps more likely amongst the youngest children [8], it would lead to an underestimate of seroconversion and seroreversion rates. Nevertheless, if trachoma control programs monitor serology with at most an annual frequency, the estimates presented in this study should be informative. Because the cohort aged over time, we cannot fully disentangle the effects of age and study month on the seroreversion rate. Finally, this study was conducted in a hyperendemic context with increasing transmission so findings may not generalize to lower transmission settings.

Trachoma programs are considering the use of serology to monitor transmission and recrudescence as populations approach elimination. In hyperendemic settings, serological measures of transmission serve as a baseline against which progress can be measured. In this longitudinal cohort, 2%–3% of seropositive children experienced seroreversion of IgG responses to chlamydia antigen Pgp3 each year, demonstrating that seroreversion is unlikely to influence estimates of seroconversion rates among young children in hyperendemic settings.

#### Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org/>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

#### Notes

**Disclaimer.** The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the National Institutes of Health or the Centers for Disease Control and Prevention. Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the US Department of Health and Human Services.

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