TITLE

Serology reflects a decline in the prevalence of trachoma in two regions of The Gambia

AUTHORS

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ABSTRACT

Trachoma is caused by *Chlamydia trachomatis* (Ct). It is targeted for global elimination as a public health problem. In 2014, a population-based cross-sectional study was performed in two previously trachoma-endemic areas of The Gambia. Participants of all ages from Lower River Region (LRR) (N = 1028) and Upper River Region (URR) (N = 840) underwent examination for trachoma and had blood collected for detection of antibodies against the Ct antigen Pgp3, by ELISA. Overall, 30 (1.6%) individuals had active trachoma; the prevalence in children aged 1–9 years was 3.4% (25/742) with no statistically significant difference in prevalence between the regions. There was a significant difference in overall seroprevalence by region: 26.2% in LRR and 17.1% in URR (p<0.0001). In children 1-9 years old, seroprevalence was 4.4% in LRR and 3.9% in URR. Reversible catalytic models using information on age-specific seroprevalence demonstrated a decrease in the transmission of Ct infection in both regions, possibly reflecting the impact of improved access to water, health and sanitation as well as mass drug administration campaigns. Serological testing for antibodies to Ct antigens is potentially useful for trachoma programmes, but consideration should be given to the co-endemicity of sexually transmitted Ct infections.

INTRODUCTION

Trachoma is caused by ocular infection with the obligate intracellular bacterium *Chlamydia trachomatis* (Ct) and is the leading infectious cause of blindness worldwide[1]. Infection is associated with clinical signs of inflammation in the conjunctiva known as active trachoma; these include trachomatous inflammation—follicular (TF) and trachomatous inflammation—intense (TI). Many repeated episodes of active trachoma over years to decades can lead to trachomatous trichiasis (TT), which may lead to impaired vision. The World Health Organization (WHO) estimates that over 200 million people in 42 countries are at risk of blindness from trachoma [2]. In 2010, approximately 1.9 million people suffered from visual impairment or blindness due to trachoma[1]. The WHO Alliance for the Global Elimination of Trachoma by 2020 (GET2020) aims to eliminate trachoma as a public health problem by 2020[2] through the SAFE Strategy (Surgery, Antibiotics, Facial cleanliness and Environmental improvement). The target is to reduce the prevalence of TF to <5% in children aged 1–9 years, and the prevalence of unmanaged TT to <0.2% in adults aged 15 years and above[3]. By 2014, seven countries had reported having met these targets nationally[4].

Evaluating the effectiveness of trachoma elimination programmes can be difficult, and current guidelines for post-intervention surveillance of Ct transmission intensity, like the elimination threshold, are based on the prevalence of TF in children aged 1–9 years. This is biologically problematic because in areas of low endemicity[5] and in populations where mass drug administration (MDA) of azithromycin has been undertaken[6], the correlation between the clinical signs of trachoma and the presence of ocular Ct infection is poor. There is therefore a need for better means by which to assess transmission.

Antibodies against *Chlamydia trachomatis* reflect cumulative exposure to Ct[7] and it has been suggested that programmes could use some measure of seroprevalence as an alternative indicator of changes in transmission[8,9]. Previous work has begun to investigate for the use of age-specific seroprevalence for surveillance in the post-MDA setting[9–11].

Serological techniques for the detection of antibodies against Ct have been used to study the epidemiology of both urogenital and ocular infections[12,13]. Recently, an enzyme-linked immunoassay (ELISA) which detects antibodies against Pgp3 (pCT03)—a highly immunogenic Ct-specific protein

encoded by the plasmid, which is highly conserved among Ct isolates[14]—has been used for analysis of samples collected from trachoma-endemic regions[15,16].

Extensive trachoma research has been undertaken in The Gambia for over 50 years, and it is therefore possible to track the declining prevalence of trachoma over time in many areas. Two National Surveys of Blindness and Low Vision demonstrated nation-wide decreases of comparable magnitude in the prevalence of active trachoma and TT between 1986 and 2000[17,18]. Specific elimination efforts, run by the National Eye Health Programme (NEHP), included mass drug administration (MDA) of azithromycin in 23 districts across the country between 2007 and 2010, including three rounds in the Lower River Region (LRR). The Partnership for the Rapid Elimination of Trachoma (PRET)[5] was embedded within the national programme and measured the prevalence of Ct infection in children residing in four districts in which MDA had been administered. MDA was not administered in URR as the TF prevalence in URR was already below the WHO threshold for elimination as a public health problem.

The aim of the current study was to use serological data from a Pgp3-specific ELISA to gain insight into the dynamics of Ct transmission in two well-characterized regions of The Gambia. The seroconversion rate (SCR)—the yearly average rate by which seronegative individuals become seropositive upon disease exposure and a surrogate for the underlying force of infection (FoI)—was estimated under different epidemiological settings in order to explore the utility of serology as a tool for surveillance in trachoma elimination programmes.

RESULTS

We recruited participants of all ages from LRR (n=1028, 41.9% male) and URR (n=840, 42.5% male). Ten participants were excluded from the study because they either declined to provide a blood sample (n=1) or had incomplete examination data (n=9). The median participant age was 13 years in LRR (range: 1-88, IQR 6–34) and 11 years in URR (range: 0–90, IQR 5–40). The proportion of participants by age group is shown in Table 1. There were significantly more females than males overall (X^2=45.332, p<0.0001), which held true in both LRR (X^2=26.483, p<0.0001) and URR (X^2=18.601, p<0.0001) and is representative of Gambian demographics[19]. Age distributions were approximately equal between the two regions, as seen in Table 1 (X^2=27.703, p=0.14).

Table 1. Age distribution of study participants, Lower River Region and Upper River Region, The Gambia, 2014.

	Both Regions		**Lower River Region**		**Upper River Region**	
	N	**%**	**N**	**%**	**N**	**%**
Overall	1868		1028		840	
Gender						
Female	1080	57.8	597	58.1	483	57.5
Male	788	42.2	431	41.9	357	42.5
Age group (years)						
1-9	742	39.7	383	37.3	359	42.7
10-19	412	22.1	231	22.5	181	21.5
20-29	191	10.2	101	9.8	90	10.7
30-39	152	8.1	79	7.7	73	8.7
40+	335	17.9	216	21.0	119	14.2

Examination findings

Thirty cases of TF were found in total (1.6%, 95% CI=1.1–2.3%), of which 25 were in children aged 1–9 years (3.4%, 95% CI=2.2–4.9, Table 2). There were 122 cases of trachomatous conjunctival scarring (TS), of which 102 were in participants aged 15 years and above (n=875, 11.6%, 95%CI=9.6–14.0); eight cases of TT, all of which were in those aged 40 years and older (0.9%, 95% CI=0.4–1.8); and one case of corneal opacity (CO), in a participant over 40 years of age (0.1%, 95% CI=0.0–0.6) (Table 2). The prevalence of TS was significantly different (X^2=4.9852, p=0.03) between LRR (78/1028, 7.6%, 95% CI=6.1–9.4%) and URR (44/840, 5.2%, 95% CI=3.9–7.0%). The TF prevalence in children aged 1–9 years was not significantly different (X^2=0.1343, p=0.71) between LRR (18, 1.8%) and URR (12, 1.4%). The prevalence of TI, TT and CO in this population was too low for further statistical analysis.

Data from this study show very low prevalence of active trachoma in these regions. In both regions, the prevalence of TF in 1–9-year-olds was below the 5% elimination threshold specified by WHO.

Table 2. Frequency of signs of trachoma in study participants, Lower River Region and Upper River Region, The Gambia, 2014.

	Frequency of signs (%)					
	N	**TF**	**TI**	**TS**	**TT**	**CO**
Overall	1868	30 (1.6)	4 (0.2)	122 (6.5)	8 (0.4)	1 (0.1)
Region						
LRR	1028	18 (1.8)	4 (0.4)	79 (7.6)	7 (0.7)	1 (0.1)
URR	840	12 (1.4)	0	43 (5.2)	1 (0.1)	0
Gender						
Female	1080	10 (0.9)	3 (0.3)	85 (7.9)	5 (0.5)	1 (0.1)
Male	788	20 (2.5)	1 (0.1)	37 (4.7)	3 (0.4)	0
Age group (years)						
1–9	742	25 (3.4)	2 (0.3)	8 (1.1)	0	0
10–19	412	4 (1.0)	1 (0.2)	10 (2.4)	0	0
20–29	191	0	0	8 (4.2)	0	0
30–39	152	1 (0.7)	1 (0.7)	15 (9.9)	0	0
40+	335	0	0	81 (24.1)	8 (2.4)	1 (0.3)
1–9 year olds -LRR	383	14 (3.7)	2 (0.5)	2 (0.5)	0	0
1–9 year olds -URR	359	11 (3.1)	0	6 (1.7)	0	0
≥10 year olds-LRR	645	4 (0.6)	2 (0.3)	76 (11.8)	7 (1.1)	1 (0.2)
≥10 year olds-URR	481	1 (0.2)	0	38 (7.9)	1 (0.2)	0

TF=trachomatous inflammation—follicular; TI=trachomatous inflammation—intense; TS=trachomatous conjunctival scarring; TT=trachomatous trichiasis; CO=corneal opacity
LRR=Lower River Region; URR=Upper River Region

Antibody responses in the populations

The threshold for seropositivity was set at the mean of the Gaussian distribution of the seronegative population plus four standard deviations, 0.810 OD_{450nm} to ensure high specificity. Previous studies using the Pgp3 ELISA have commonly used a threshold set as three standard deviations above the

mean of the negative population (the 97.5% confidence interval) [15,20,21]. Using that lower threshold resulted in the same qualitative conclusions being drawn (See Supplementary Information).

The seroprevalence of antibodies against Pgp3 for each region, by age and gender, is summarised in Table 3. The overall seroprevalence in LRR and URR was 26.2% (95% CI=23.5–29.0) and 17.1% (95% CI=14.7–19.9%), respectively. There was a significant difference in overall seroprevalence between the two regions (X^2=20.72, p<0.0001). Figure 1 shows the seroprevalence by age group and region. In children 1-9 years old, seroprevalence was 4.4% in LRR and 3.9% in URR. As expected, the prevalence of anti-Pgp3 antibodies increased with age using the non-parametric test for trend (z-score=23.35, p<0.0001). The seroprevalence doubled between 10–19-year-olds and the next oldest age group, 20–29-year-olds, both in the two regions combined, and in each region. Across both regions, in study participants who had no signs of trachoma, seroprevalence was 21% (95% CI = 19–23). Of those who had active trachoma (TF and/or TI) (n=30), 3% were seropositive (95% CI = 0.1–17) and of those who had scarring trachoma (TS and/or TT and/or CO in either eye; n=122) 56% were seropositive (95% CI = 44–67).

Table 3. Seroprevalence of anti-Pgp3 antibodies by region, gender and age, Lower River Region and Upper River Region, The Gambia, 2014.

	Both regions combined			Lower River Region			Upper River Region		
		Prevalence			Prevalence			Prevalence	
	N	%	95%CI	N	%	95%CI	N	%	95% CI
Overall	1868	22.1	20.2-24.0	1028	26.2	23.5-29.0	840	17.1	14.7-19.9
Gender									
Female	1080	27.4	24.8-30.2	597	32.0	28.3-35.9	483	21.7	18.1-25.7
Male	788	14.8	12.4-17.5	431	18.1	14.6-22.1	357	10.9	7.9-14.6
Age group (years)									
1–9	742	4.2	2.9-5.9	383	4.4	2.6-7.0	359	3.9	2.1-6.5
10–19	412	11.7	8.7-15.1	231	12.5	8.6-17.5	181	10.5	6.4-16.0
20–29	191	25.1	19.1-31.9	101	27.7	19.3-37.5	90	22.2	14.1-32.2
30–39	152	48.0	39.9-56.3	79	51.9	40.4-63.3	73	43.8	32.2-55.9
40+	335	63.3	57.9-68.5	216	70.8	64.3-76.8	119	49.6	40.3-58.9

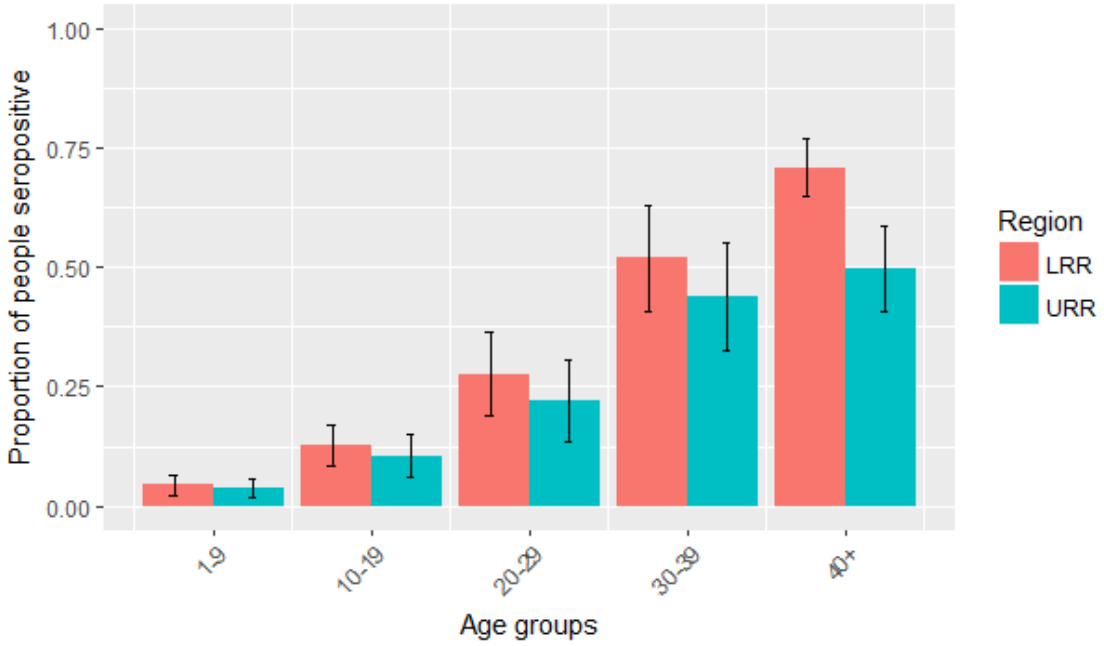

Figure 1. Proportion of participants who were seropositive for anti-Pgp3 antibodies by age group and region, Lower River Region (LRR) and Upper River Region (URR), The Gambia, 2014. Vertical bars indicate 95% CIs.

The prevalence of antibodies was significantly higher in females than in males (z-score=6.384, p<0.0001). The same was true in each region (LRR: z-score=4.881, p<0.0001; URR: z-score=4.114, p<0.0001). When data were considered for each age group, the seropositivity difference between males and females was only significant for 10–19-year-olds (z-score=2.667, p=0.0077) and 30–39-year-olds (z-score=0.2551, p=0.0107). Seroprevalence by gender and age group is shown in Figure 2.

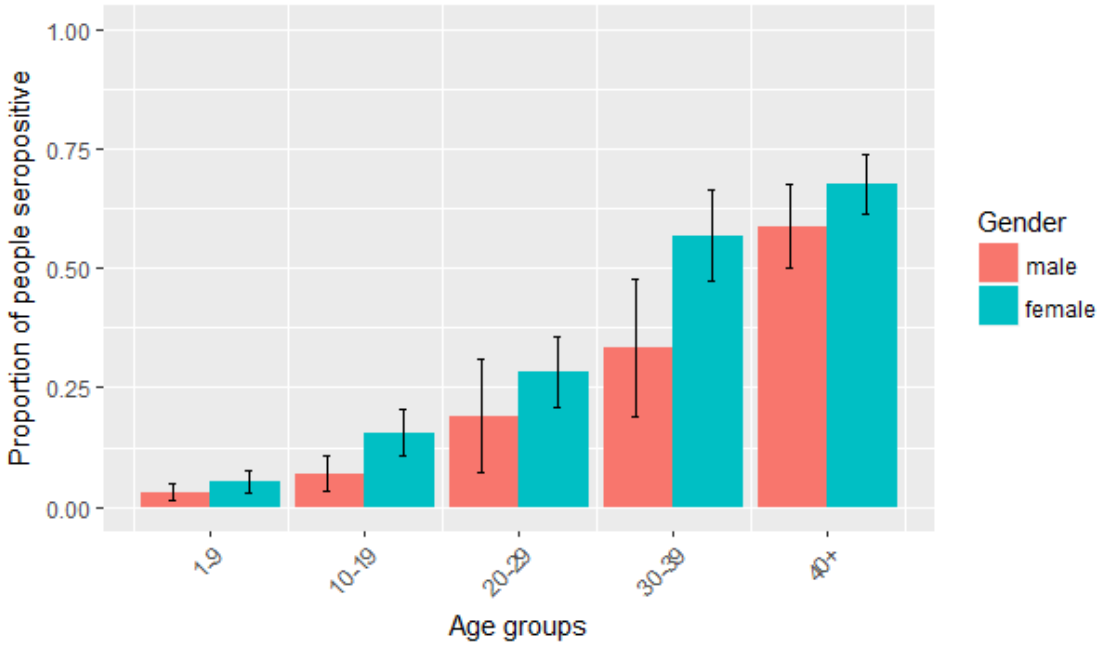

Figure 2. Proportion of participants who were seropositive for anti-Pgp3 antibodies, by age group and gender, Lower River Region and Upper River Region, The Gambia, 2014. Vertical bars indicate 95% confidence intervals.

Reduction in seroconversion rates over time

We used a reversible catalytic model together with the profile likelihood method to identify reductions in seroconversion rates (SCR) in both LRR and URR. The reversible catalytic model is based on the premise that individuals transit between seropositive and seronegative states with specific average rates[22]. Abrupt reductions in SCRs were identified in both LRR and URR (Table 5 and Figure 3). These changes in SCR are estimated to have occurred 23 and 19 years before data collection in LRR and URR, respectively (Figure 3). For LRR, the profile likelihood plot (Figure 3) could be cautiously interpreted to indicate that an additional, smaller change in SCR occurred approximately 10 years ago, which coincides with the azithromycin MDA undertaken between 2007 and 2010[5]. For LRR, the SCR would appear to have dropped from an incidence of 0.062 yearly events per person, to 0.010 yearly events per person. These estimates implied a putative 6.2-fold decrease in transmission intensity. In URR, the estimate of the past SCR dropped from 0.050 yearly events per person to 0.008 yearly events per person, a 6.3-fold decrease (Table 5). Figure 4 shows the expected seroprevalence curves as function of age assuming a change in transmission intensity.

Table 5. Maximum likelihood estimates for the past and current seroconversion and seroreversion rates (SCR and SRR, respectively) associated with data collected from participants in Lower River Region and Upper River Region, The Gambia, 2014 where the respective 95% confidence intervals are shown in brackets. P-values <0.05 are indicative of a change in transmission intensity when comparing two reversible catalytic models, one assuming constant and stable transmission over time and another assuming an abrupt reduction in transmission intensity somewhere in the past.

Region	SCR_{past}	$SCR_{current}$	SRR	Fold change	p-value
Lower River Region	0.062 (0.038, 0.103)	0.010 (0.008, 0.013)	0.009 (0.004,0.018)	6.2	<0.001
Upper River Region	0.050 (0.020, 0.123)	0.008 (0.006, 0.012)	0.019 (0.008, 0.046)	6.3	<0.001

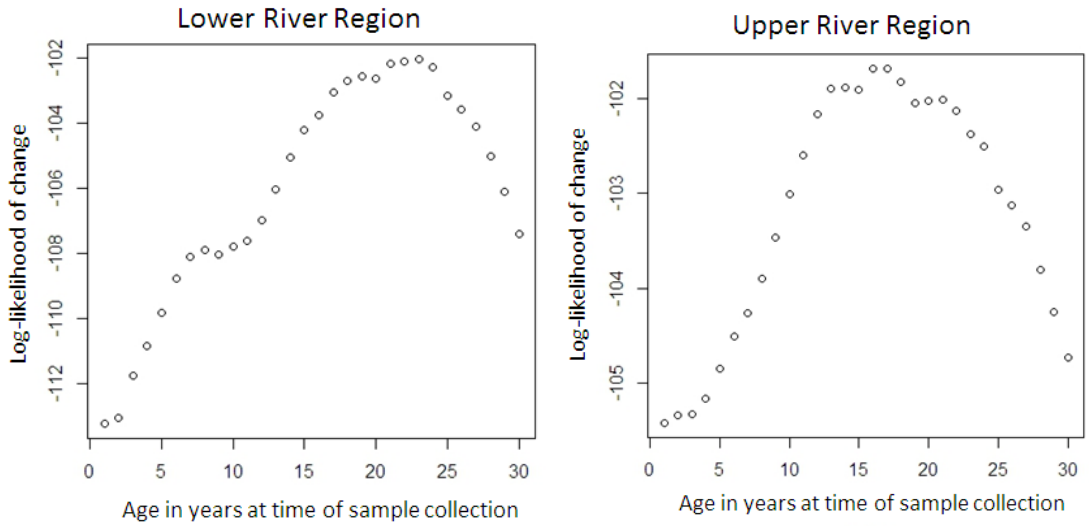

Figure 3. Profile likelihood plots for antibodies against Pgp3 in Lower River Region (LRR) and Upper River Region (URR), The Gambia, 2014. Profile likelihood plots show the log likelihood of a reversible catalytic model allowing for an abrupt change in SCR occurring at iterative years, with the maximum being the time point at which that change is most likely to have occurred. The plot of LRR (left) indicates one major peak 23 years prior to sample collection. The plot of URR (right) suggests a change 19 years prior to sample collection.

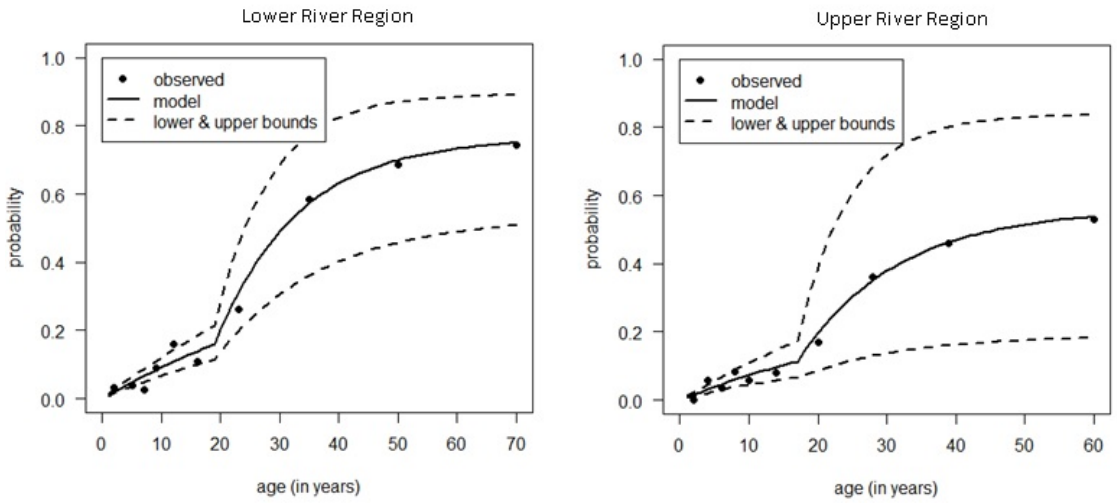

Figure 4. Predicted age-seroprevalence curves for antibodies against Pgp3, Lower River Region and Upper River Region, The Gambia, 2014, where the dots represent the observed seroprevalences when the age distribution was broken down in ten-year age groups. Models allowing for a change in transmission intensity at a point in time were fitted.

Evidence for elimination

Data from this study show very low prevalence of active trachoma in these regions. In children aged 1–9 years, only 4.2% had detectable antibodies against Pgp3. In both regions, the prevalence of TF in 1–9-year-olds was below the 5% elimination threshold specified by WHO.

DISCUSSION

This study examines the age-specific prevalence of anti-Pgp3 antibodies in two of The Gambia's eight regions. One of the regions (LRR) had received azithromycin MDA for trachoma from 2007–2010, whilst the other (URR) had not because the TF prevalence in URR was already below the WHO threshold for elimination as a public health problem. We have demonstrated significant changes in SCR that would be consistent with either (a) a significant drop in the transmission intensity of ocular Ct in the mid to late 1990s or (b) a confounding signal, manifesting as a continuous process of seroconversion, which likely results from exposure to urogenital infection with Ct following sexual debut. It is possible that the data reflect both things, but without current data describing the population prevalence of urogenital and ocular infections, the proportional contributions of STIs and trachoma to the SCR cannot be fully assessed.

The WHO's guidelines for validation of elimination of trachoma as a public health problem focus programmatic attention on the prevalence of TF in children aged 1–9 years and TT in people aged 15 years and older. The Gambian government is currently compiling evidence for validation of trachoma elimination, following completion of its three-year TF/TT surveillance plan, which began in 2011[23,24]. Previous studies have demonstrated declines in the prevalence of active trachoma in The Gambia both prior to[6,25] and in response to the implementation of specific trachoma-control interventions[26–28]. Increased access to water, education and healthcare in The Gambia during recent decades are thought to have had an impact[29], manifesting in a secular decline. Regardless of cause, the prevalence of active trachoma in 0–14-year-olds fell from 10.4% in 1986[18] to less than 5% in 1996[17], and has subsequently remained low. Prevalence data from historical studies is presented in Table 6. More recently, six years before the survey reported here, communities in LRR received azithromycin to treat ocular Ct infection, further reducing the prevalence of trachoma in this region[5,24], while communities in URR did not. Although not measured in our study, previous work has shown that there has been a reduction in the prevalence of ocular Ct infection in two villages in LRR[30,31] with the most recent measurements showing 0.5% infection prevalence in PRET villages, a portion of which are in LRR[5]. In line with the findings of those previous studies, we provide a further data point showing TF prevalences <5% for each region. The seroprevalence estimates and FoI modelling performed here are measures of trachoma transmission intensity that strongly support The Gambia's claim to have eliminated trachoma from these areas.

Table 6. Previously published data on the prevalence active trachoma and trachomatous trichiasis, Lower River Region and Upper River Region, The Gambia, 1986–2013. Data from the 1986 survey was not available for 0–9 year olds, thus we have used the data for 0–14 year olds.

Region	Prevalence of signs of trachoma				
	1986[18]	1996[18]		2013[24]	
	TF/TI (0–14 year-olds)	TF/TI (0–9 year-olds)	TT (≥30 year-olds)	TF (0–9 year-olds)	TT (≥15 year-olds)
Lower River Region	12.3%	11.5%	4.6%	1.8%	1.0%
Upper River Region	5.0%	1.3%	1.3%	0.4%	0.07%

TF = trachomatous inflammation - follicular, TI = trachomatous inflammation - intense, TT = trachomatous trichiasis

Our analysis of age-specific seroprevalence suggests that the FoI is currently very low, with a substantial decrease in SCR in children having occurred approximately 20 years ago (compared to the children that grew up before then), or a substantial increase in SCR occurring now in those aged around 20 years (compared to their contemporary juniors). The change is too acute to reflect secular decline in the FoI of trachoma. If it is not related to transmission of urogenital Ct, it could be interpreted to reflect the impact of the door-to-door distribution of topical 1% tetracycline ointment across the country, which took place in the late 1990s[32].

The antigenic overlap between ocular Ct infection and sexually transmitted infections with the same organism is a potential barrier to making correct inferences about ocular Ct transmission intensity from anti-Pgp3 seroprevalence data. The urogenital Ct consideration is extremely important. No currently available serological test distinguishes between exposure to ocular and urogenital CT infection. The inflections in the SCR curves (Figure 4) could be signals related to seroconversion in response to acquisition of urogenital infection following sexual debut. Interestingly, the seroprevalence among 10–19-year-old Gambian females in our study was almost double that of their male counterparts of the same age. In the Gambia, the median age at which females first have sex is 18.6 years, with 52% of women aged 20–24 years surveyed as part of the Demographic and Health Survey (2013) having had sexual intercourse by age 20; in males the median age is 23.1 years, with 48% of men aged 25–29 years surveyed having had sexual intercourse by age 22[33]. This could in part explain why we observed a gender difference in seroprevalence in the 10–19-year-old age range, as those with earlier sexual debut would be expected to be more likely to acquire STIs[34–36].

Conversely, in a population in which the urogenital Ct infection prevalence has been consistently low, it might be expected that anti-Pgp3 serological data more accurately reflect longitudinal trends in ocular Ct transmission. Whilst data suggest that the prevalence of urogenital Ct infections has historically been very low in rural areas of The Gambia[37,38], there are no recent data, nor data based on modern molecular testing methods. A 2003 study from Malicounda in Thiès Region of neighbouring Senegal showed the prevalence of urogenital Ct infection there to be 0.3% (n=73)[39]. Additionally, a systematic review of global estimates of incidence and prevalence of sexually transmitted infections (STI), including urogenital chlamydia, estimated the prevalence of urogenital chlamydia to be 2.9% in low-income countries[40] such as The Gambia, although it is noted that this study did not include data from The Gambia.

For the Lower River Region, the profile likelihood plot (Figure 3) could be cautiously interpreted to indicate that a second, smaller change in SCR occurred approximately 10 years ago, a time frame that coincides with the azithromycin MDA undertaken between 2007 and 2010[5]. A similar secondary maximum was not observed in the plot for URR, where MDA was not given. Given the contrast between LRR and URR, it seems plausible that this change in FoI reflects the impact of the NEHP's azithromycin MDA, which would have reduced the risk of acquiring either ocular[41] or urogenital Ct[42]. Any impact of MDA that could be inferred from the increase in profile likelihood at 7-10 years in LRR is small by comparison to the process of change in FOI that peaked some two decades ago and which probably relates to longer-term health and social improvements in the Gambia.

The confidence intervals associated with SCRs seen in Figure 4 are very broad (which in part reflects the uncertainty of modelling approaches) and comparison between the charts for LRR and URR is indicative but not conclusive of a difference in SCR between the two regions. Although a larger sample size would reduce the uncertainty, interpretation of the model depends to a large extent on

the magnitude of the change as well as the timing between the change in SCR and sample collection, as seen in malaria modelling work[21]. The very large sample sizes required of studies that could delineate SCR changes with high precision could be prohibitive.

A recent serological study using samples from Tanzania[9] examined the age-specific seroprevalence of anti-Pgp3 antibodies in a trachoma-endemic community that had received two rounds of high coverage azithromycin MDA. As a result of that intervention, the all-ages prevalence of ocular Ct infection had fallen from 9.5% to 0.1% two years after MDA[43], and to 0% five years after MDA[44], with a corresponding 11-fold decrease in SCR[9]. This change in infection prevalence occurred in a more defined (and probably narrower) timeframe than the one we have studied in The Gambia. This resulted in a more acute change in SCR, as has been demonstrated in malaria modelling exercises[45,46]. Previous trachoma modelling studies suggest that an individual may require upwards of 100 lifetime ocular Ct infections in order to develop TT[47], so even a modest reduction in transmission may have significant public health implications and reduce the future incidence of TT.

Research is ongoing to address remaining challenges in interpreting trachoma seroprevalence. It is unclear how many infections are required for seroconversion to occur. Studies involving urogenital Ct infection suggest 68% of infected women produce antibodies against Ct[48]. The intensity of the inflammatory response, and the surface area of inflamed mucosa, however, are both likely to differ between the infected conjunctiva and infected female urogenital tract. Additionally, further work is needed to determine the half-life of Pgp3 antibodies and seroreversion rates. A previous study that examined a high-prevalence community before and after 1 round of azithromycin suggests that individual anti-Pgp3 antibody levels decreased slightly six months after drug treatment, but not enough to be considered seroreversion[49]. This is similar to results seen for urogenital Ct infection, where anti-Pgp3 antibody titres decreased over 4–7 years, but patients were still in the seropositive range[50]. Although we have estimated SRR in this study (Table 5), a more accurate estimate could be obtained from a longitudinal study collecting serum samples over a period of years. In low transmission settings, such as post-MDA communities, the SRR may be under-estimated if the assumption of a balance between total number of seropositive and seronegative individuals does not hold true. Further studies to determine SRR are currently underway.

Although dichotomising antibody levels to a simple seropositive/seronegative classification provides a straightforward estimation of seroprevalence, SCR estimates could potentially be improved by using a model based on antibody levels and multiple sampling time points, as suggested by Yman *et al*[51]. Such models might assume that antibody levels increase with age, as exposure is age-dependent and that transmission intensity can be calculated by measuring the boost in antibody levels. The use of age group-specific geometric mean antibody levels could be explored[51], in addition to SCR.

Approximately 4% of 1–9-year-olds were positive for antibodies to Pgp3, which may be due to previously acquired ocular Ct infections and/or to ocular or respiratory Ct infections acquired at birth from mothers with urogenital Ct infections[52]. This seroprevalence is within the range of prevalence values previously estimated in post-MDA surveys in Tanzania and Nepal [8,9]. There was also no observed increase in anti-Pgp3 antibody positivity with age in 1–9-year-olds (Supplementary Table 2), in contrast to what is observed in trachoma-endemic settings, whether treatment-naive [11] or

after 3 rounds of MDA [10,15]. Focusing on age-specific changes in seropositivity as a measure of cumulative exposure to ocular Ct infection might offset antibody responses from peri-natal infection, as the latter would be expected to be consistent across all ages, or even to decline with increasing age. The data from The Gambia presented here, combined with those from a variety of pre- and post-MDA settings, contribute to an understanding of the potential use of antibody-based surveillance of children to ensure a lack of infection recrudescence.

Data from paired pre- and post-MDA surveys would substantially improve modeling efforts to describe changes over time in. The inclusion of infection data for both ocular and urogenital Ct infection are needed to further clarify how urogenital Ct contributes to observed seropositivity rates.

METHODS

Ethical Review

This study was conducted in accordance with the Declaration of Helsinki. It received approval from the London School of Hygiene & Tropical Medicine Ethics Committee (LSHTM; reference 7059) and The Gambia Government/Medical Research Council Joint Ethics Committee (SCC1408v2). CDC investigators did not engage with study participants.

Survey methodology

We conducted a population-based, cluster-random-sampled survey was conducted in February-March 2014. The Gambia is divided into geographically-defined census Enumeration Areas (EAs) of approximately 600-800 people each. Sampling by EA is equivalent to sampling settlements with probability proportional to their size[53]. Twenty EAs in each of URR and LRR were randomly selected for participation. Trained field workers sensitised villagers and obtained verbal community-consent from each village chief (*alkalo*). Field workers and *alkalos* compiled a list of households for each EA, from which households were randomly selected for census and recruitment. The study and consent form were explained to the head of each selected household and prospective participants. All members of selected households were invited to participate, regardless of age. Written (thumbprint or signature) consent was obtained from each participant aged ≥18 years, while a parent or guardian provided written consent for each participating child aged under 18 years. Children aged 12-17 years provided assent before participating.

The trachoma graders were experienced in field grading for active trachoma and had received regular training according to PRET[5] and Global Trachoma Mapping Project (GTMP) protocols[54,55]. After informed consent was received, the grader examined both eyes of the subject using a binocular loupe (2.5×) and a torch. The grader changed gloves between each participant to minimise the risk of carry-over infection. In accordance with the Gambian NEHP policy, antibiotics were provided to individuals with evidence of active trachoma and to residents of their household.

Each participant had a finger-prick blood sample collected onto filter paper (Trop-Bio, Townsville, Australia) using a sterile single-use lancet (BD Microtrainer, Dublin, Ireland). Each filter paper had six extensions, calibrated to absorb 10 µL of blood each. Samples were air-dried for approximately five

hours and then placed in individual Whirl-Pak plastic bags (Nasco, Modesto, California) which were stored with desiccant sachets (Whatman, Little Chalfont, UK) at -20°C. All samples were shipped to LSHTM for testing.

ELISA Assay

Dried blood spots (DBS) were tested for antibodies against Pgp3 according to the method previously described[15]. Briefly, serum was eluted from dried blots spots then applied to a plate coated with Pgp3 protein[10]; known standards were included and assayed in triplicate on each plate. Following incubation, bound antibody was detected with HRP-labelled mouse anti-human IgG(Fc)-HRP (Southern Biotech, Birmingham, USA). Plates were incubated and washed, and then TMB (KPL, Gaithersburg, USA) was added to develop the plates. The reaction was stopped with 1N H_2SO_4 and optical density was read at 450 nm (OD_{450}) on a Spectramax M3 plate reader (Molecular Devices, Wokingham UK). Readings were corrected for background by subtracting the average absorbance of three blank wells containing no serum, using Softmax Pro5 software (Molecular Devices).

Statistical Methods

Blanked OD_{450} values for samples were normalised against the 200 U standard included on each plate[15]. Laboratory work was undertaken masked to demographic and clinical information. Statistical analyses were carried out using R[56]. Using the "survey" package and assuming a design effect of 2.65[54], the 95% confidence intervals (CI) were calculated using the Clopper-Pearson interval[57]. Wilcoxon-Mann-Whitney z-scores[58] were calculated to compare the proportion seropositive between different regions, ages, and genders. The non-parametric test for trend was used to measure the increase in prevalence of anti-Pgp3 antibodies with age.

A finite mixture model[21] was used to classify the samples as seropositive or seronegative based on normalised OD_{450} values. The data were fitted using maximum likelihood methods, estimating the distribution parameters for each classification group (seropositive or seronegative) as well as the proportion of samples in each category to fit the overall distribution of results[59]. To ensure that the assay had high specificity, the threshold for seropositivity was set using the mean of the Gaussian distribution of the seronegative population plus four standard deviations (the quantile inclusive of 99.994%) of the seronegative population[21,59].

Population age groups were categorized according to known time points of changes in disease prevalence. The youngest age group was 1–9-year-olds, who in LRR are likely to have been born during or after MDA with azithromycin. The oldest group included people aged 40 years and above, who experienced secular declines in trachoma prevalence prior to 1986, the year of the first National Survey of Blindness and Low Vision[18]. Participants aged between 10 and 39 years were grouped into 10-year categories.

Maximum likelihood methods were used to fit a simple reversible catalytic model (assuming a constant transmission intensity over time, and that people enter into the model as seronegative) to the seropositivity data[22]. The model is described as function of SCR (the mean rate by which seronegative individuals become seropositive upon disease exposure) and a SRR (the mean rate by which seropositive individuals revert to a seronegative status in the absence of re-infection). SCR is usually interpreted as a proxy of the FoI for each region. As there are no long-term data regarding

the SRR for Ct, we allowed it to vary as determined by the model. Age of each individual was rounded to the nearest integer in order to generate age-specific seroprevalence curves for each region. A second set of curves was generated using a model allowing for a sudden change in SCR, as would be seen if there were a change in the FoI, due to an intervention, for example. Both models assume the age distribution of the populations match the typical age distribution of Africa. Wilks' likelihood ratio test was used to compare the single-FoI model to the model allowing a change in SCR at some point in time. P-values<0.05 indicated that the latter model described the data better than the single-FoI model. Profile likelihood plots were generated to determine the most likely timing of the change in FoI as described elsewhere[21].

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AUTHOR CONTRIBUTIONS

S.J.M., R.B., D.C.W.M., A.W.S. and C.h.R designed the study and wrote the paper, D.L.M., G.C., S.G. C.h.R. and S.J.M. developed the protocol for serological analysis, S.J.M. performed serological analysis, S.J.M., C.h.R and N.S. performed the statistical analysis, R.B. trained the trachoma graders, S.J.M., H.J., P.M., R.B., S.E.B., D.C.W.M. and C.h.R. organized field data collection. H.J. and P.M. performed the field grading of trachoma. H.P. provided critical feedback on the paper. All authors reviewed and approved the manuscript.

ADDITIONAL INFORMATION

Sarah Gwyn is employed by the commercial company IHRC, Inc. and is a contractor at the Centres for Disease Control and Prevention.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.