Re-emergence of *Chlamydia trachomatis* infection after mass antibiotic treatment of a trachoma-endemic Gambian community: a longitudinal study

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Lancet 2005; 365: 1321–28

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Summary

Background **Community-wide mass antibiotic treatment is a central component of trachoma control. The optimum frequency and duration of treatment are unknown. We measured the effect of mass treatment on the conjunctival burden of *Chlamydia trachomatis* in a Gambian community with low to medium trachoma prevalence and investigated the rate, route, and determinants of re-emergent infection.**

Methods **14 trachoma-endemic villages in rural Gambia were examined and conjunctival swabs obtained at baseline, 2, 6, 12, and 17 months. Mass antibiotic treatment with azithromycin was given to the community at baseline. *C trachomatis* was detected by qualitative PCR and individual infection load then estimated by real-time quantitative PCR.**

Findings **C *trachomatis* was detected in 95 (7%) of 1319 individuals at baseline. Treatment coverage was 83% of the population (1328 of 1595 people). The effect of mass treatment was heterogeneous. In 12 villages all baseline infections (34 [3%] of 1062 individuals) resolved, and prevalence (three [0·3%]) and infection load remained low throughout the study. Two villages (baseline infection: 61 [24%] of 257 individuals) had increased infection 2 months after treatment (74 [30%]), after extensive contact with other untreated communities. Subsequently, this value reduced to less than half of that before treatment (25 [11%]).**

Interpretation **Mass antibiotic treatment generally results in effective, longlasting control of *C trachomatis* in this environment. For low prevalence regions, one treatment episode might be sufficient. Infection can be reintroduced through contact with untreated populations. Communities need to be monitored for treatment failure and control measures implemented over wide geographical areas.**

Introduction

Trachoma is the leading infectious cause of blindness worldwide.[1] Recurrent episodes of chronic follicular conjunctivitis (clinically active trachoma), caused by *Chlamydia trachomatis*, promote the development of conjunctival scarring, entropion, trichiasis, and ultimately blinding corneal opacification. Trachoma is a major public-health problem affecting some of the world's poorest regions. 146 million people are estimated to have active trachoma.[1] WHO and its partners are promoting the SAFE strategy (surgery for trichiasis, antibiotics for infection, facial cleanliness, and environmental improvements to reduce transmission of the organism) to control blinding trachoma.[2]

Trachoma control programmes use antibiotics to reduce the burden of *C trachomatis* infection in endemic communities. However, both infection and disease are frequently recorded in previously treated populations. The determinants of re-emergent infection are poorly understood. The infection could arise either through failure of treatment to clear infection or from reinfection after successful treatment. Primary treatment failure could result from an ineffective drug or incomplete treatment course. Reinfection could arise through contact with untreated individuals from within or outside the community, and in the case of topical antibiotics, autoreinfection can take place from untreated extraocular sites, such as the nasopharynx.[3] After *C trachomatis* has been reintroduced into a treated community, various factors affect the ease with which it spreads between individuals: availability of water and sanitation, activities of eye-seeking flies, and density of living conditions.[4]

Some of these difficulties can be overcome by community-wide treatment with the oral antibiotic azithromycin.[5,6] The drug is well tolerated and very effective against *C trachomatis*. It is given as one supervised dose, so compliance is high. Children are usually given a weight-based dose of azithromycin suspension, although evidence suggests that height-based dosing with tablets can also be used.[7] Treatment of all members of a trachoma endemic community, irrespective of their clinical phenotype, allows individuals harbouring clinically inapparent infection to be treated.[8,9] Unfortunately, even with very wide treatment coverage, infection frequently re-emerges.[6] Therefore, to improve the long-term effectiveness of trachoma control, repeated antibiotic treatment is advocated.[2] WHO is developing recommendations on the frequency and duration of treatment; however, there

are few data on which to base such recommendations for different severities of endemic trachoma.

In a region with low to medium prevalence trachoma, we measured the effect of mass azithromycin treatment on the community burden of *C trachomatis* infection. The community was then followed to assess the rate, routes, and determinants of re-emergent infection.

Methods

The study was approved by the Gambian government/UK Medical Research Council joint ethics committee (scientific coordinating committee number 856) and the ethics committee of the London School of Hygiene and Tropical Medicine, UK. Informed consent took place at three levels: village, family, and individual. Documented informed consent from the head of every family was required for enrolment in the study.

Community survey

The study took place in a cluster of villages in Upper Saloum district, The Gambia.[8] Village surveys and a census were undertaken. Individuals who were residents in the study area for at least 6 months of the year were enrolled. Visitors were excluded. Data were obtained for latrine access, water supply, livestock, house construction, and other socioeconomic indicators. The age, sex, ethnic group, education, and sleeping room of all individuals were recorded. Throughout the study, a fieldworker made weekly visits to every compound. The census was updated and the destination and duration of travel out of the study area were recorded. New residents were enrolled if they intended to stay for at least 6 months. Origin and duration of stay of any visitors were recorded.

Clinical assessment

Clinical assessment was done at baseline and repeated at 2, 6, 12, and 17 months after the treatment. Examinations from all villages were synchronous, being done within 6 to 10 days for every time point. A fieldworker documented ocular or nasal secretions and any fly-eye contact during the minute before examination. An ophthalmologist examined the left eye of every patient for trachoma, which was classified by use of the WHO trachoma grading system.[10] Clinically active trachoma was defined as either a follicular score of two or three (F2/3) or a papillary score of three (P3). These scores are equivalent to follicular trachomatous inflammation (TF) and intense trachomatous inflammation (TI) of the WHO simplified trachoma grading system, respectively.[11]

We anaesthetised conjunctivas with proxymetacaine 0·5% eye drops (Minims, Chauvin Pharmaceuticals, Romford, UK). For DNA isolation, a Dacron polyester-tipped swab (Hardwood Products Company, Guilford, ME, USA) was used in a standardised manner to obtain a sample from the upper tarsal conjunctiva and placed in a dry polypropylene tube. All samples were kept on ice packs until transfer to a –20°C freezer later the same day.

Antibiotic treatment

Immediately after the baseline clinical assessment, all participants were offered antibiotic treatment. Children (aged <16 years) were given one oral dose of azithromycin (suspension, 20 mg/kg, up to 1 g). Infants younger than 6 months were given tetracycline eye ointment (1%, twice daily for 6 weeks). Adults were given one oral dose of azithromycin (tablets, 1 g), apart from women of childbearing age who received oral erythromycin (500 mg, twice daily for 2 weeks). The examination and treatment of each village lasted between a half and 2 days, dependent on size, and all villages were treated within 9 days during April, 2001.

Qualitative and quantitative PCR for *C trachomatis*

DNA was extracted from swab samples and tested by qualitative PCR for *C trachomatis*, by use of the Amplicor CT/NG kit (Roche Molecular Systems, Branchburg, NJ, USA).[8,9] All Amplicor testing was done at the MRC Laboratories, The Gambia. Positive specimens by the Amplicor CT/NG test were analysed further with a quantitative real-time PCR for the chlamydial *omp1* gene.[8,9] Briefly, a sample of the Amplicor CT/NG DNA extract for every positive sample was purified and concentrated by use of the QIAamp DNA Mini Kit (Qiagen, Crawley, UK).

Baseline and 2-month samples were assayed on a LightCycler (Roche Diagnostics, Lewes, UK) at the London School of Hygiene and Tropical Medicine, and the 6, 12, and 17-month samples assayed on an ABI 5700 sequence detection system (Applied Biosystems, Warrington, UK) in The Gambia. Both systems used the same primer sequences, thermal cycle conditions, and standards diluted from the same stock.[8,9] The reactions taking place on the ABI 5700 machine had a total volume of 25 μL, and contained 5 μL of DNA extract, 12·5 μL of SYBR green PCR master mix (Quantitect, Qiagen), 5·5 μL of water, and 1 μL of a 10 μM solution of each primer.

Statistical analysis

Data were analysed in STATA version 7 and S-PLUS version 6.1. The estimated quantified load of infection is expressed as the number of copies of *omp1* per swab and is the geometric mean of two replicate assays. To follow changes in infection for the entire or subsections of the study population, we calculated adjusted geometric means of the infection load. Since these groups included some uninfected individuals, a value of one was added to the quantification results of infected individuals, and a value of one ascribed to individuals with a negative qualitative PCR result. The geometric mean was calculated and then a value of one was subtracted from the result. This measure, also known as the Williams mean, is used when the data contain one or more zero values, in which case the true geometric mean is always zero, and therefore of restricted utility.[12]

	Entire study area		12 villages		Villages 1 and 3	
	Disease	Infection	Disease	Infection	Disease	Infection
Baseline	103 (8%)	95 (7%)	60 (6%)	34 (3%)	43 (17%)	61 (24%)
2 months	79 (6%)	76 (6%)	42 (4%)	2	37 (15%)	74 (30%)
6 months	68 (5%)	44 (3%)	32 (3%)	2	36 (13%)	42 (15%)
12 months	38 (3%)	31 (3%)	14 (2%)	6 (1%)	24 (11%)	25 (11%)
17 months	47 (4%)	28 (2%)	25 (3%)	3	22 (9%)	25 (11%)

Data are number (%).

Table 1: **Prevalence of clinically active disease and *C trachomatis* infection at every assessment**

Multivariable logistic regression models for infection at every time point were developed, with adjustment for compound level clustering by generalised estimating equations.[8] For the 17-month follow-up, a robust cluster model was used. A maximum likelihood estimate was made for samples identified as positive by Amplicor (the most sensitive assay in the study) but negative by quantitative PCR.[8,9] For samples assayed on the LightCycler, the maximum likelihood estimate was 5·7 copies of *omp1* per swab for one negative replicate, and 4·1 copies of *omp1* per swab if both replicates were negative. For samples assayed on the ABI 5700, the maximum likelihood estimate for one negative replicate was 4·5 copies of *omp1* per swab, and for two negative replicates the value was 3·4 copies of *omp1* per swab.

A two-part model was used to estimate infection load as a function of age, including the zero values. A logistic regression quadratic in log-age was used for the probability of samples being positive, and of the positive cases, log-quantifications were modelled by linear regression. Both components were fitted simultaneously by maximum likelihood. We estimated the age-specific mean as the fitted probability of samples being positive, multiplied by the fitted mean of the positives.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Our study population lived in a cluster of 14 villages; in small subsistence farming communities with a mean population of 115 (SD 69). At baseline, 1595 people lived in the area, of which 1319 (83%) were examined and sampled. During the 17-month follow-up, 109 individuals were born, 26 died, 137 moved into the study area, and 240 moved away. Total population size remained stable throughout the study (1585 [at 2 months], 1600 [6 months], 1653 [12 months], 1575 [17 months]). And at follow-up assessments, a reasonable proportion of the population was examined; 1344 (85%), 1333 (83%), 1108 (67%), and 1210 (77%) at 2, 6, 12, and 17 months, respectively. The main reason for individuals not being examined was travel out of the study area. The population consisted of the Wolof (881, 55%) and Fula (714, 45%) ethnic groups, living in separate villages. Villages 1 and 3 were both entirely Wolof.

Antibiotic treatment was given to 1328 (83%) individuals of the total population and to 766 (89%) of 863 children. Azithromycin was used for 1079 (81%) of 1328 treatments. In villages 1 and 3, treatment was given to 143 (86%) of 167 individuals and 118 (92%) of 128, respectively. Untreated individuals were generally older (geometric mean 15·6 years, 95% CI 13·6–18·0) and more often male (153, 57%), than those who received antibiotics (geometric mean 11·6 years, 95% CI 10·9–12·4; male individuals, 597, 44%).

Overall prevalence of *C trachomatis* infection (determined by qualitative PCR) and clinically active trachoma were similar to each other and fell gradually during the course of the study (table 1). The pretreatment prevalence of disease and infection for each of the

	Active disease (%)	Infection (%)
Baseline/2 months		
–/–	1040 (90%)	1030 (89%)
+/–	47 (4%)	60 (5%)
–/+	24 (2%)	39 (3%)
+/+	45 (4%)	27 (2%)
Total	1156	1156
2 months/6 months		
–/–	1078 (91%)	1092 (92%)
+/–	48 (4%)	57 (5%)
–/+	31 (3%)	20 (2%)
+/+	28 (2%)	16 (1%)
Total	1185	1185
6 months/12 months		
–/–	887 (92%)	908 (94%)
+/–	39 (4%)	23 (2%)
–/+	13 (1%)	17 (2%)
+/+	21 (2%)	12 (1%)
Total	960	960
12 months/17 months		
–/–	828 (94%)	848 (96%)
+/–	17 (2%)	15 (2%)
–/+	21 (2%)	6 (1%)
+/+	17 (2%)	14 (2%)
Total	883	883

+=PCR positive or active trachoma. –=PCR negative or clinically normal. Only individuals seen at both consecutive assessments were included. Infection status was determined by qualitative PCR.

Table 2: **Changes in active disease and infection status between consecutive pairs of assessments**

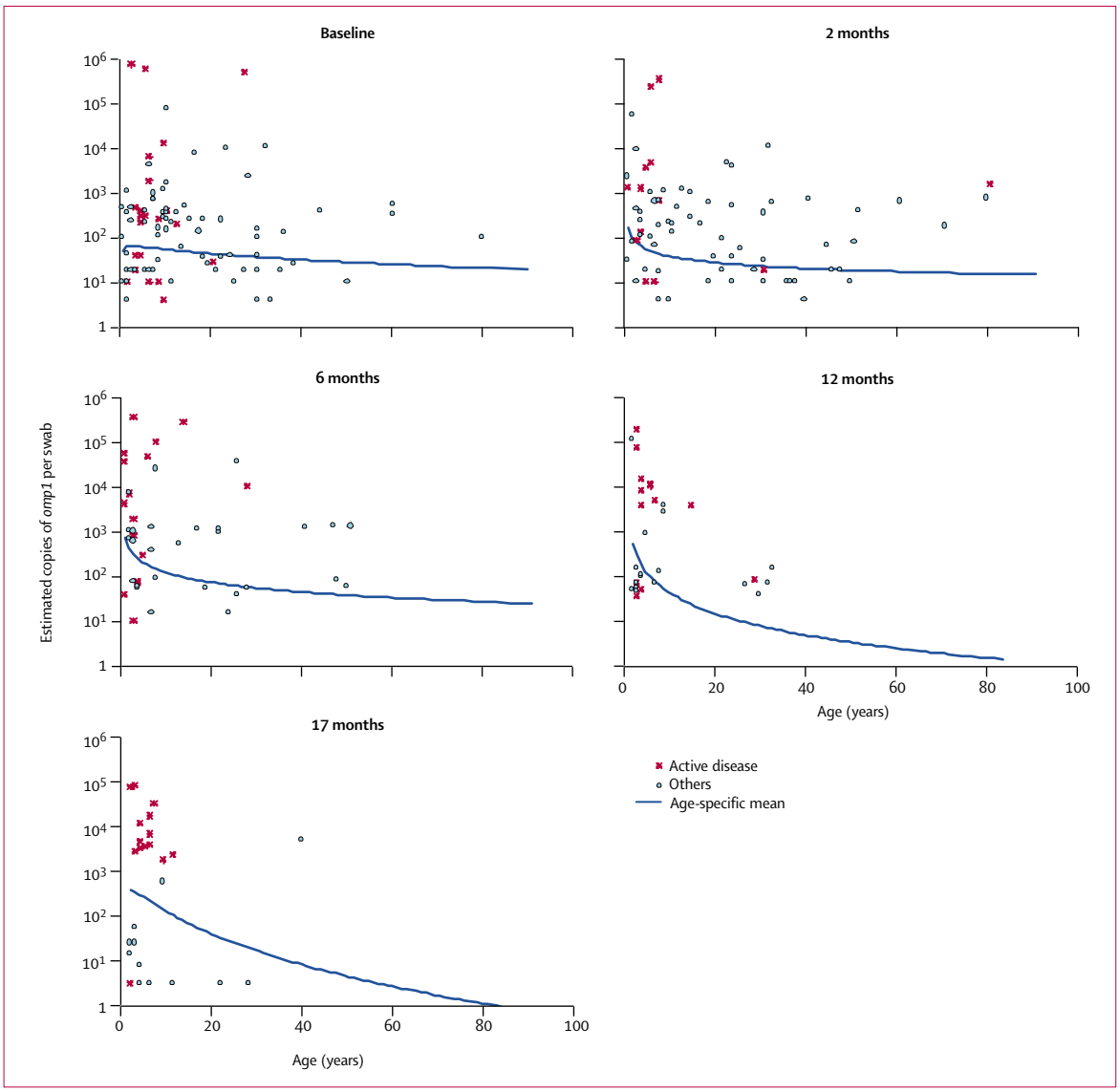

Figure: **Distribution of estimated quantified load of *C trachomatis* infection at baseline and at follow-up assessments**

14 villages has been previously described.[8] Response to antibiotic treatment was heterogeneous. 12 villages (n=1062 at baseline) had a substantial reduction in infection prevalence by 2 months, with all baseline cases of infection resolving, irrespective of infection load (table 1). These 12 villages included those with the highest pretreatment disease (village 6) and infection prevalence (village 14). During the 17-month follow-up, only a few isolated infection episodes took place. By contrast, the prevalence of infection in villages 1 and 3 (n=257 at baseline; table 1) increased 2 months' post-treatment, despite better antibiotic coverage. Subsequently, prevalence fell to below half pretreatment values.

Across the study area, many individuals changed their infection and clinical status between consecutive assessments (table 2). 2 months after treatment, 60 (70%) of 87 patients infected at baseline were no longer infected, whereas 39 (59%) of 66 infected at 2 months were new cases. These proportions of infection status changes were similar to those recorded during subsequent follow-up. At 2 months, *C trachomatis* infection was detected in six (3%) of 183 previously untreated individuals, compared with 70 (6%) of 1161 treated individuals. Response of infection to different antibiotic regimens used in this study did not differ significantly.

C trachomatis infection load was quantified in 269 (99%) of 273 samples identified as positive by qualitative PCR. Four samples had insufficient volume for testing, one each from baseline, 2, 6, and 17 months. For every time point, distribution of the estimated number of *omp1* copies per swab was skewed; most patients had low loads (figure). The number of individuals who were identified

	Individuals identified as positive by Amplicor*	Individuals identified as positive by quantitative PCR*	Entire population †	Villages 1+3†	All other villages†	Active disease†
Baseline	169	199	0·45	2·61	0·16	2·77
2 months	186	231	0·34	3·63	0·01	2·04
6 months	879	879	0·25	1·77	0·01	5·82
12 months	620	620	0·20	1·14	0·03	13·96
17 months	400	1575	0·14	0·94	0·00	11·70

*Geometric mean. †Adjusted geometric mean (calculations also included uninfected individuals).

Table 3: **Geometric mean or adjusted geometric mean of *C trachomatis* infection load for all assessments**

as positive by Amplicor (ie, qualitative PCR) but negative by the quantitative PCR assay was 4, 3, 0, 0, and 6, for baseline, 2, 6, 12, and 17 months, respectively. From the 2-month follow-up onwards, 166 (93%) of 179 detected infections were in villages 1 or 3. Before treatment and at 2 months, the age-specific mean of quantified load of infection did not decline with age (figure). However, at subsequent follow-up assessments, events of infection became increasingly concentrated in children, and the age-specific mean infection load decreased with age.

Of all infected individuals, including those identified as positive by qualitative PCR but negative by quantitative PCR, the geometric mean estimated that the *omp1* copy number increased and then decreased during the study (table 3). When those identified as positive by qualitative PCR but negative by quantitative PCR were

	Baseline		2 months		6 months		12 months		17 months	
	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
Univariate analysis										
Individual factors										
Age 0–4 years	1·00	..	1·00	..	1·00	..	1·00	..	1·00	..
Age 5–9 years	1·20 (0·69–2·09)	0·525	1·13 (0·58–2·22)	0·723	0·35 0·15–0·81)	0·0140	0·36 (0·15–0·87)	0·0242	0·75 (0·32–1·76)	0·508
Age 10–14 years	1·02 (0·57–1·82)	0·959	0·54 (0·24–1·22)	0·139	0·13 (0·03–0·56)	0·0062	0·08 (0·01–0·57)	0·0121	0·26 (0·06–1·13)	0·072
Age >15 years	0·68 (0·39–1·18)	0·171	0·98 (0·53–1·81)	0·955	0·30 (0·15–0·61)	0·0007	0·18 (0·07–0·45)	0·0003	0·16 (0·05–0·49)	0·0013
Sex (female)	0·96 (0·63–1·46)	0·851	0·71 (0·45–1·13)	0·151	1·84 (0·96–3·56)	0·068	1·13 (0·54–2·35)	0·748	1·13 (0·52–2·42)	0·763
Ethnic origin (Wolof=0, Fula=1)	0·67 (0·43–1·04)	0·079	0	<0·0001	0·03 (0·00–0·22)	0·0005	0	<0·0001	0·04 (0·01–0·32)	0·0021
Travel after last assessment	..	..	79·9 (25·0–255)	<0·0001	1·46 (0·67–3·20)	0·341	0·86 (0·35–2·12)	0·743	0·54 (0·19–1·58)	0·261
Visitors (none *vs* any)	..	..	1·72 (1·03–2·86)	0·0370	0·19 (0·08–0·43)	<0·0001	1·12 (0·45–2·76)	0·807	1·21 (0·51–2·88)	0·664
Clinical findings										
Active disease (TF, TI, or both)	4·57 (2·71–7·69)	<0·0001	3·76 (1·97–7·17)	<0·0001	13·6 (6·92–26·6)	<0·0001	30·4 (13·4–68·7)	<0·0001	34·8 (15·4–78·9)	<0·0001
Follicular trachoma (F2/3 or TF)	4·49 (2·64–7·62)	<0·0001	3·41 (1·72–6·79)	0·0005	15·8 (7·98–31·3)	<0·0001	34·6 (15·1–79·3)	<0·0001	40·1 (17·3–93·1)	<0·0001
Intense trachoma (P3 or TI)	12·7 (5·03–32·1)	<0·0001	5·36 (1·71–16·9)	0·0041	3·72 (0·46–30·4)	0·220	53·0 (11·3–249)	<0·0001	17·6 (4·41–70·3)	<0·0001
Ocular secretions	2·77 (1·49–5·12)	0·0012	23·4 (5·13–106)	<0·0001	47·0 (7·65–289)	<0·0001	26·4 (7·04–99·0)	<0·0001	15·1 (2·90–78·3)	0·0012
Nasal secretions	1·70 (1·00–2·87)	0·0467	2·50 (1·03–6·07)	0·0437	4·19 (1·41–12·5)	0·0100	15·9 (5·74–44·0)	<0·0001	17·7 (5·27–59·7)	<0·0001
Fly-eye contact	2·50 (1·38–4·54)	0·0025	7·97 (3·88–16·3)	<0·0001	9·96 (4·04–24·6)	<0·0001	52·8 (11·3–247)	<0·0001	109 (35·7–331)	<0·0001
Infection load >200 *omp1* copies per swab at previous assessment	..	..	6·61 (3·17–13·8)	<0·0001	13·9 (5·95–32·3)	<0·0001	72·3 (26·9–194)	<0·0001	430 (83–2221)	<0·0001
Clustering/crowding markers										
Other individual with active disease living in room	3·19 (2·06–4·95)	<0·0001	2·22 (1·30–3·78)	0·0035	7·74 (4·17–14·4)	<0·0001	7·68 (3·54–16·6)	<0·0001	9·59 (4·43–20·7)	<0·0001
Other individual with active disease in compound	10·9 (4·40–27·1)	<0·0001	12·7 (4·62–35·0)	<0·0001	4·56 (2·02–10·3)	0·0002	4·07 (1·80–9·18)	0·0007	6·73 (2·54–17·8)	0·0001
Residence in village 1 or 3	9·4 (6·02–14·7)	<0·0001	230 (55·8–943)	<0·0001	94·2 (22·6–391)	<0·0001	18·3 (7·30–45·1)	<0·0001	38·8 (6·92–42·2)	<0·0001
Environmental factors										
No latrine access	19·4 (7·07–53·1)	<0·0001	32·3 (7·89–132)	<0·0001	15·9 (3·84–66·1)	0·0001	4·01 (1·53–10·5)	0·0048	∞	<0·0001
Cows staying in compound	3·15 (1·99–4·99)	<0·0001	2·08 (1·28–3·37)	0·0030	3·29 (1·65–6·56)	0·0007	3·67 (1·57–8·60)	0·0027	3·11 (1·31–7·38)	0·0099
Multivariable logistic regression models*										
Age 0–4 years	1·00	..	1·00	..	1·00	..	1·00	..	1·00	..
Age 5–9 years	1·09 (0·69–1·73)	0·715	1·28 (0·76–2·16)	0·359	0·40 (0·21–0·77)	0·0062	0·47 (0·26–0·85)	0·0128	1·03 (0·29–3·71)	0·961
Age 10–14 years	0·95 (0·63–1·45)	0·828	0·63 (0·31–1·28)	0·199	0·16 (0·04–0·66)	0·0112	0·08 (0·01–0·80)	0·0312	0·72 (0·13–3·87)	0·700
Age >15 years	0·89 (0·46–1·73)	0·724	0·78 (0·49–1·25)	0·299	0·49 (0·24–1·03)	0·059	0·31 (0·13–0·74)	0·0077	0·27 (0·02–3·38)	0·308
Active disease (TF, TI, or both)	2·79 (1·38–5·62)	0·0041	1·02 (0·50–2·08)	0·965	5·07 (1·77–14·6)	0·0025	6·75 (1·77–25·7)	0·0052	25·7 (10·1–65·6)	<0·0001
Other individual with active disease in compound	17·7 (4·80–65·8)	<0·0001	5·03 (0·96–26·2)	0·055	3·85 (0·94–15·8)	0·062	2·14 (0·61–7·47)	0·235	1·27 (0·24–6·80)	0·779
Ocular secretions	1·69 (0·76–3·76)	0·194	2·76 (0·18–41·4)	0·461	4·91 (0·47–55·0)	0·183	4·79 (1·37–16·7)	0·0139	2·06 (0·52–8·05)	0·301
No latrine access	13·3 (4·01–44·1)	<0·0001	6·00 (0·69–51·9)	0·103	9·73 (1·18–80·5)	0·0348	1·18 (0·28–4·93)	0·824	∞	<0·0001
Infection load >200 *omp1* copies per swab at previous assessment	..	..	0·99 (0·38–2·61)	0·988	3·44 (0·71–16·7)	0·125	31·7 (8·62–117)	<0·0001	81·4 (13·6–487)	<0·0001
Travel after last assessment	..	..	12·2 (7·38–20·2)	<0·0001	0·96 (0·22–4·23)	0·960	1·51 (0·49–4·62)	0·472	0·37 (0·11–1·33)	0·128

TF=follicular trachomatous inflammation. TI=intense trachomatous inflammation. *Models adjusted for compound level clustering by generalised estimated equations.

Table 4: **Analysis of associations between *C trachomatis* infection and various factors at every assessment**

excluded, the geometric mean infection load rose towards the end of the study (table 3). Across the entire community, the mean infection load declined with every successive follow-up. For villages 1 and 3, the infection load was highest at 2 months and subsequently fell, and for the other 12 villages, it dropped to a very low value after treatment (table 3).

Associations between various risk factors and *C trachomatis* infection were assessed for every time point by univariate analysis and multivariable logistic regression models, adjusted for compound level clustering by generalised estimating equation (table 4). The 14 villages were analysed together, because there was no previous basis for stratification. Results with p values lower than 0·05 were regarded as significant. Several variables had odds ratios tending towards either zero or infinity. All villages are within a confined, continuous, geographical area and seem homogeneous. Infection was not related to age before treatment or at 2 months; however, from 6 months onwards, infection prevalence declined with increasing age.

Neither Wolof nor Fula ethnic groups were associated with increased risk of infection before treatment. After treatment, we recorded a univariate association between infection and Wolof ethnic origin. This association arose because most post-treatment infections were confined to two Wolof villages (1 and 3). Ethnic origin was excluded from the multivariable logistic regression analysis because of colinearity. Clinically active trachoma was associated with infection at all time points, except at 2 months. Cohabitation in the same compound as another individual with clinically active trachoma was associated with increased risk of infection before treatment. Other clinical markers such as ocular and nasal secretions were not independently associated with infection. Residence in a compound without a pit latrine was associated with infection at baseline, 6 months, and 17 months.

Detection of *C trachomatis* at 12 months and 17 months was strongly associated with individuals having had a high infection load (ie, >200 copies of *omp1* per swab) at the preceding assessment (table 4). This high threshold divides the infection load distribution at each time point into two groups of about equal size. Travel outside the study area between the baseline and 2-month assessments was a strong risk factor for infection at 2 months. Individuals hosting visitors were not associated with an increased risk of infection.

During the first month after antibiotic treatment, 376 travelling events outside the study area were recorded compared with an mean of 85 (SD 27·6) for all other months. People from villages 1 and 3 made 295 (78%) of 376 journeys during the first month, by contrast with only 290 (21%) of 1360 during the remainder of the study, which coincided with their proportion in the total population (295, 18%). The geometric mean age of travellers was younger during the first month (14·3 years, 95% CI 12·8–16·1) than those travelling during the remaining 16 months of the study (20·3 years, 19·0–21·7). Towards the end of the first month, almost all the residents (including children) of villages 1 and 3 went on a pilgrimage to Touba in Senegal.

Discussion

In our study, mass azithromycin treatment of *C trachomatis* infection was followed by longlasting control in this trachoma-endemic community. However, the initial effect was less than we anticipated; although there was a small reduction in infection prevalence and load 2 months after treatment (by contrast with large reductions in prevalence of infection previously reported[6]), response to treatment was heterogeneous. In 12 villages, all cases of *C trachomatis* infection before treatment resolved by 2 months, irrespective of infection load. During the rest of the study only a very few sporadic cases were detected and the community load remained very low. By contrast, in two villages (1 and 3) infection increased at 2 months, despite higher antibiotic coverage, and then fell well below pretreatment values. Infection was associated with active disease, apart from at 2 months. Other risk factors for infection were travel outside the study area, high infection load at the previous assessment, residence in a compound without a latrine, and cohabitation with a person with active trachoma.

The increased prevalence of infection 2 months after treatment in villages 1 and 3 was probably due to reinfection during attendance of the annual pilgrimage at Touba, Senegal. This event attracts about a million people from across the subregion. Accommodation is basic and crowded with restricted access to water and sanitation. Survey data indicate that the prevalence of active trachoma in Senegal is at least twice that in The Gambia,[13] and still higher rates have been reported in neighbouring Mauritania, Mali, and Guinea.[14,15]

By contrast with the pattern of travel generally seen during the study, many children accompanied their parents, and would have been able to mix with children from infected communities elsewhere in the subregion, helping the dissemination of infection across national borders. Coordinated programme planning is needed to combat this potential for reinfection. The elimination of blinding trachoma in this subregion (and other similar areas) will probably need implementation of control measures across wide geographical areas and international borders.

It is unlikely that untreated individuals living in villages 1 and 3 contributed much to the reinfection of treated individuals, because treatment coverage was high and infection prevalence was lower in untreated than in treated individuals at 2 months. Could the antibiotic have been ineffective against the strains of *C trachomatis* circulating in these two villages? This possibility is also unlikely in view of the success of treatment in the other 12 villages, and because there has

been no convincing published evidence of azithromycin resistance in *C trachomatis*.

Infection prevalence declined in villages 1 and 3 from 2 months onwards, which indicated that in this environment *C trachomatis* infection is not in stable equilibrium. Various factors may affect how readily infection is transmitted after reintroduction into a successfully treated community. At several time points, residence in a compound without a pit latrine was associated with an increased risk of infection. The eye-seeking fly *Musca sorbens* probably contributes to the transmission of trachoma in this region.[16] The use of pit latrines might restrict available breeding sites in the domestic environment, reducing the fly population and therefore the transmission of *C trachomatis*.[17]

There is also evidence from more arid areas than that assessed in our study linking scarcity of water to trachoma.[4] Water was available throughout the year from wells within a few minutes' walk of every compound. This availability might have helped suppress transmission, perhaps through increased use of water for washing faces and fomites, such as bed linen.[18] The downward trend seen in this study is consistent with changes throughout The Gambia in recent years, and coincides with improvement in water supply to rural communities.[15] These findings lend support to the implementation of the facial cleanliness and environmental improvement components of the SAFE strategy to sustain long-term trachoma control.

Additionally, in areas of low prevalence of trachoma, there may be only one or a very few strains of *C trachomatis* circulating. With time, much of the community will be able to develop protective immune responses, which resolve infection. In the absence of new strains of *C trachomatis*, the infection prevalence would be expected to decline and become increasingly confined to young children in whom resolution of infection is usually delayed.[19]

Towards the end of this study, a small group consisting mainly of children emerged who were infected at consecutive time points with high infection loads. This shift underlies the finding that among infected individuals, there was an increasing trend in the mean infection load (table 3) as *C trachomatis* became increasingly confined to a small group of children who seemed to have difficulty in controlling the infection. The finding of individuals who were susceptible to persistent infection and may be at greater risk of developing scarring disease has been reported in other endemic areas.[20]

We recorded a significant association between disease and infection at each time point, except at 2 months after treatment. Recent antibiotic treatment weakens the association between disease and infection because clinical signs persist after infection has cleared.[19] The reintroduction of infection into villages 1 and 3 at 2 months resulted in the infection of many adults (figure), in whom the typical features of active trachoma rarely develop in response to infection.[19] Towards the end of the study, the association between disease and infection strengthened because infection became largely confined to young children who readily develop clinical disease and resolved in adults who are usually clinically normal.

Trachoma control programmes do not usually have access to diagnostic tests for *C trachomatis* and rely on clinical signs to identify individuals or groups likely to harbour infection. Overall, across the entire study area, the prevalence of active trachoma indicated that of infection. However, as we and other studies have shown, these clinical signs are often not reliable markers of infection on an individual basis.[8,19,21–23] Before treatment, there were several villages where active trachoma but not infection was common.[8]

The situation is complicated with the introduction of antibiotic treatment. In low to medium prevalence regions where infection is not in stable equilibrium, the relation between signs and infection might vary substantially, dependent on the degree of pooling of infection in children, as was noted in this study. The development of an affordable, simple field test for infection would aid trachoma control programmes operating in low prevalence areas in establishing which settlements should be treated and in rapidly identifying communities with re-emergent infection (such as villages 1 and 3 in our study).

Repeated mass antibiotic distribution is advocated to control trachoma.[2] Only few empirical data are available to guide the frequency of re-treatment. A mathematical model exploring this issue indicated that for regions with more than 50% active disease in children, treatment would be needed every 6–12 months, whereas for areas with moderate prevalence (<35% in children) re-treatment every 1 or 2 years might be sufficient.[24] No projections were made for areas with low prevalence. The model assumed 100% treatment coverage across a very wide geographical area, precluding reinfection from other communities; however, such conditions are unlikely to be achieved.

Our data indicate that in communities with a low to medium prevalence, longlasting control of *C trachomatis* infection can be achieved with one dose of azithromycin. This finding is consistent with a previous study of mass antibiotic treatment in this area.[6,25] Our data also indicate that the effect of treatment should be monitored since substantial reinfection can arise. Local knowledge about potentially relevant sources of reinfection, such as the one recorded in this study, is important and should be factored into the planning of treatment and follow-up. Therefore, control programmes operating in low prevalence areas should undertake clinical assessments of previously treated communities yearly or every 2 years. If clinical signs of the disease are still evident, repeated mass treatment should be considered. The use of field tests for infection would be a good means to guide this strategy.

Conflict of interest statement

Our group has received research grants for separate studies from the International Trachoma Initiative (ITI), which was set up by the Edna McConnell Clark Foundation and Pfizer (the manufacturers of azithromycin). However, neither ITI or Pfizer have had access to our results, nor have they seen or attempted to influence the writing of this report.

Contributors

All investigators contributed to the design and implementation of the study, and to the writing or editing of the report. M Burton, R Bailey, and P Makalo undertook the fieldwork. M Holland developed the quantitative PCR assay. E Aryee did the Amplicor testing. M Burton, R Bailey, and N Alexander undertook the analysis. D Mabey coordinated the project.

Acknowledgments

We thank the people of the Jareng villages for their good humoured participation in this study; and the members of the field team for help with collection of data and samples. The Wellcome Trust/Burroughs Wellcome Fund provided financial support for this study. Pfizer, the manufacturer, donated the azithromycin.

References

1 Thylefors B, Negrel AD, Pararajasegaram R, Dadzie KY. Global data on blindness. *Bull World Health Organ* 1995; **73:** 115–21.

2 World Health Organization. Future approaches to trachoma control. Report of a global scientific meeting. WHO//PBL/96·56. Geneva: World Health Organization, 1997.

3 West S, Munoz B, Bobo L, et al. Nonocular Chlamydia infection and risk of ocular reinfection after mass treatment in a trachoma hyperendemic area. *Invest Ophthalmol Vis Sci* 1993; **34:** 3194–98.

4 Emerson PM, Cairncross S, Bailey RL, Mabey DC. Review of the evidence base for the "F" and "E" components of the SAFE strategy for trachoma control. *Trop Med Int Health* 2000; **5:** 515–27.

5 Bailey RL, Arullendran P, Whittle HC, Mabey DC. Randomised controlled trial of single-dose azithromycin in treatment of trachoma. *Lancet* 1993; **342:** 453–56.

6 Schachter J, West SK, Mabey D, et al. Azithromycin in control of trachoma. *Lancet* 1999; **354:** 630–35.

7 Munoz B, Solomon AW, Zingeser J, et al. Antibiotic dosage in trachoma control programs: height as a surrogate for weight in children. *Invest Ophthalmol Vis Sci* 2003; **44:** 1464–69.

8 Burton MJ, Holland MJ, Faal N, et al. Which members of a community need antibiotics to control trachoma? Conjunctival *Chlamydia trachomatis* infection load in Gambian villages. *Invest Ophthalmol Vis Sci* 2003; **44:** 4215–22.

9 Solomon AW, Holland MJ, Burton MJ, et al. Strategies for control of trachoma: observational study with quantitative PCR. *Lancet* 2003; **362:** 198–204.

10 Dawson CR, Jones BR, Tarizzo ML. Guide to Trachoma Control. Geneva: World Health Organization, 1981.

11 Thylefors B, Dawson CR, Jones BR, West SK, Taylor HR. A simple system for the assessment of trachoma and its complications. *Bull World Health Organ* 1987; **65:** 477–83.

12 Kirkwood BR. Essential Medical Statistics, 1st edn. Oxford: Blackwell Science, 1988.

13 World Health Organization. Report of the sixth meeting of the WHO Alliance for the Global Elimination of Blinding Trachoma. Geneva: World Health Organization, 2001.

14 Moalic E, Dueymes JM, Baron R, Le Flohic AM. Cross-sectional survey of trachoma in school age children in the region of Thies (Senegal). *Pediatr Infect Dis J* 2000; **19:** 979–83.

15 Dolin PJ, Faal H, Johnson GJ, Ajewole J, Mohamed AA, Lee PS. Trachoma in The Gambia. *Br J Ophthalmol* 1998; **82:** 930–33.

16 Emerson PM, Lindsay SW, Walraven GE, et al. Effect of fly control on trachoma and diarrhoea. *Lancet* 1999; **353:** 1401–03.

17 Emerson PM, Lindsay SW, Alexander N, et al. Role of flies and provision of latrines in trachoma control: cluster-randomised controlled trial. *Lancet* 2004; **363:** 1093–98.

18 West S, Munoz B, Lynch M, et al. Impact of face-washing on trachoma in Kongwa, Tanzania. *Lancet* 1995; **345:** 155–58.

19 Bailey R, Duong T, Carpenter R, Whittle H, Mabey D. The duration of human ocular *Chlamydia trachomatis* infection is age dependent. *Epidemiol Infect* 1999; **123:** 479–86.

20 Smith A, Munoz B, Hsieh YH, Bobo L, Mkocha H, West S. *OmpA* genotypic evidence for persistent ocular *Chlamydia trachomatis* infection in Tanzanian village women. *Ophthalmic Epidemiol* 2001; **8:** 127–35.

21 Ward M, Bailey R, Lesley A, Kajbaf M, Robertson J, Mabey D. Persisting inapparent chlamydial infection in a trachoma endemic community in The Gambia. *Scand J Infect Dis Suppl* 1990; **69:** 137–48.

22 Baral K, Osaki S, Shreshta B, et al. Reliability of clinical diagnosis in identifying infectious trachoma in a low-prevalence area of Nepal. *Bull World Health Organ* 1999; **77:** 461–66.

23 Bird M, Dawson CR, Schachter JS, et al. Does the diagnosis of trachoma adequately identify ocular chlamydial infection in trachoma-endemic areas? *J Infect Dis* 2003; **187:** 1669–73.

24 Lietman T, Porco T, Dawson C, Blower S. Global elimination of trachoma: how frequently should we administer mass chemotherapy? *Nat Med* 1999; **5:** 572–76.

25 Fraser-Hurt N, Bailey RL, Cousens S, Mabey D, Faal H, Mabey DC. Efficacy of oral azithromycin versus topical tetracycline in mass treatment of endemic trachoma. *Bull World Health Organ* 2001; **79:** 632–40.