Which Members of a Community Need Antibiotics to Control Trachoma? Conjunctival Chlamydia trachomatis Infection Load in Gambian Villages

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PURPOSE. Trachoma is the leading cause of infectious blindness worldwide. Control strategies target antibiotic therapy to individuals likely to be infected with Chlamydia trachomatis on the basis of clinical signs. However, many studies have found chlamydial infection in the absence of clinical disease. It has been unclear whether such individuals represent a significant reservoir of infection. In the current study, a quantitative polymerase chain reaction (PCR) assay was used to investigate the distribution and determinants of chlamydial infection load in an endemic community, and the findings were used to evaluate the potential effectiveness of different control strategies.

METHODS. Members of a trachoma-endemic community (n = 1,319) in a rural area of The Gambia were examined for signs of disease, and tarsal conjunctival swab samples were collected. C. trachomatis was initially detected by qualitative PCR. The load of infection was then estimated by real-time quantitative PCR.

RESULTS. Chlamydial infection was detected in 7.2% of the population. The distribution of infection load was skewed, with a few individuals having high loads. Only 24% of infected individuals had signs of active trachoma. Infection loads were higher in those with clinically active disease and were highest among those with severe inflammatory trachoma. High infection loads were associated with having no accessible latrine and living with a person with active disease.

CONCLUSIONS. In this low-prevalence setting, infected individuals without signs of active trachoma constitute a significant reservoir of infection. Treatment of a defined unit of people who live with someone with clinically active trachoma would effectively target antibiotic treatment to infected people without signs of disease. (Invest Ophthalmol Vis Sci. 2003;44:4215–4222) DOI:10.1167/iovs.03-0107

Trachoma is the leading cause of infectious blindness in the world.1 Recurrent infection by Chlamydia trachomatis, the causative organism, results in chronic follicular conjunctivitis. Conjunctival scarring ensues, which leads to entropion, trichiasis, and, ultimately, blinding corneal opacification. In the absence of effective control programs, it has been suggested that there could be a doubling of trachoma-related blindness by 2020.2 Therefore, the World Health Organization with its partners in the Alliance for the Global Elimination of Trachoma are promoting the SAFE Strategy (surgery for trichiasis, antibiotics for infection, facial cleanliness, and environmental measures to reduce transmission) to control trachoma.3

The “A” component of the SAFE Strategy seeks to deliver effective antibiotic therapy to infected individuals within endemic communities to reduce transmission of chlamydia and hence the long-term risk of blindness. Several topical and systemic antibiotics have been used against trachoma.4 Results have often been disappointing, with clinical disease reemerging soon after treatment. This has been attributed to factors such as poor compliance with prolonged topical or systemic treatment, limited drug efficacy, and reinfection from extracoronal sites or untreated individuals. Some shortcomings of topical antibiotics have been overcome by use of the azalide antibiotic azithromycin.5,6 It is well tolerated and is given in a single supervised oral dose, and so compliance is high, and extracorneal sites of infection are treated. The drug is reliably absorbed and highly effective against chlamydia. After the formation of the Azithromycin Donation Program through the International Trachoma Initiative, the use of this antibiotic in trachoma control programs has become feasible in some endemic countries. However, uncertainty remains over the most effective way to use antibiotics to help eliminate trachoma as a blinding disease.

Currently, trachoma control programs use clinical signs to prioritize individuals and communities for treatment. Several approaches have been advocated, including mass treatment of entire communities (irrespective of the individual’s clinical status) as well as strategies that target “high-risk” groups such as families with active cases.7 However, the signs of active trachoma do not always correlate well with the presence of chlamydial infection. There are individuals with clinical signs of active disease in whom the organism cannot be detected and, conversely, clinically normal people in whom C. trachomatis is found.6,8,9 It has been suggested that the major reservoir of chlamydial infection in endemic communities is young children, particularly those with intense disease.10 However, the extent to which clinically normal yet infected individuals represents a source of C. trachomatis infection is unknown.

The answer to this question may have significant implications for trachoma control programs. If such individuals were found to constitute a significant reservoir of infection then not treating them may compromise the effectiveness of antibiotic distribution.


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To investigate whether clinically normal but infected individuals represent a significant reservoir of infection, we conducted a community-wide survey. Chlamydial infection was detected by qualitative PCR of conjunctival swab samples. Quantitative real-time PCR was used to estimate the number of copies of the *C. trachomatis omp1* gene in infected samples. Results were related to clinical signs of trachoma and potential predisposing factors for disease and infection.

**METHODS**

**Community Survey**

This study was conducted in a rural population from a trachoma-endemic area of Upper Saloum District, The Gambia. The study villages and their constituent compounds were surveyed. A census was conducted. Individuals were enrolled if they were normally resident in the study area. Personal data included name, age (in years), sex, ethnic group, education, and sharing of the bedroom. Data were also collected on water supply, latrine access, bathroom construction materials, livestock, and other socioeconomic indicators.

**Clinical Assessment**

A field worker documented the presence of any ocular or nasal secretions and any fly–eye contact during the minutes before the clinical examination. The left eye was examined by an ophthalmologist (MJB) for signs of trachoma and graded using the WHO Trachoma Grading System. In this system the component signs are graded separately: follicles, papillary hypertrophy and inflammation, conjunctival scarring, corneal scarring, and entropion/trichiasis. Individuals were considered to have clinically active trachoma if there was either a follicular score of 2 or 3 (F2/3) or a papillary score of 3 (P3). These are equivalent to Trachomatous Inflammation, Follicular (TF) and Trachomatous Inflammation, Intense (TI) of the WHO Simplified Trachoma Grading System, respectively. The conjunctiva was anesthetized with proxymetacaine 0.5% eye drops (Minims; Chauvin Pharmaceuticals, Romford, UK). A single, sterile Dacron polyester-tipped swab (Hardwood Products Company, Guilford, ME) was used to collect a sample from the upper tarsal conjunctiva in a standardized fashion. Four horizontal passes of the swab were made across the conjunctiva, with a quarter turn of the swab after each pass. Swabs were placed in dry polypropylene tubes and kept in a cool box with ice packs for several hours before storage in a −20°C freezer. Care was taken to avoid cross-contamination by ensuring that the swab came into contact only with the conjunctiva and that the examiner’s hands were washed between subjects. Compounds were revisited to meet individuals who were not initially seen. The reason for no examination was recorded.

**Qualitative PCR for *C. trachomatis***

Conjunctival samples were tested for *C. trachomatis* with a PCR-based assay, according to the manufacturer’s instructions (Amplipicr CT/NG Test; Roche Molecular Systems, Branchburg, NJ). This assay is for a conserved 201-nucleotide sequence on the cryptic plasmidial plasmid. DNA was extracted by vortexing the swabs in 500 μL of CT/NG lysis buffer for 20 seconds followed by adding 500 μL of CT/NG specimen diluent. A combination of 50 μL of DNA extract and 50 μL of CT/NG master mix was used in the amplification reaction, and the plates were read (Dias plate reader (Dynex Technologies, Chantilly, VA). Samples were considered positive if the A520 optical density (OD) ≥ 0.8 or more, equivocal at 0.2 ≤ OD ≤ 0.8 and negative at OD < 0.2. Equivocal samples were retested in duplicate. A parallel internal control plate assessed PCR inhibition. Inhibited samples were retested after 20 μL of DNA extract had been diluted with 80 μL of a 50:50 mixture of CT/NG lysis buffer and CT/NG specimen diluent.

**Quantitative PCR for *C. trachomatis***

The load of *C. trachomatis* DNA was estimated by a real-time quantitative PCR assay for *omp1*, a single-copy gene found on the chlamydial chromosome. Only Amplipicr-positive specimens and those equivocal on two or more occasions were analyzed in the second assay. The Amplipicr lysis buffer was incompatible with the quantitative PCR assay. Therefore, the DNA from a 360-μL aliquot of the original Amplipicr DNA extract was purified and concentrated to a volume of 50 μL, using a kit (QIAamp DNA Mini Kit; Qiagen, Crawley, UK) according to the manufacturer’s instructions. A thermocycler (LightCycler; Roche Diagnostics, Mannheim, Germany) was used to perform the quantitative PCR assay in 20-μL glass capillary tubes. Each reaction contained 4 μL of DNA extract, 10 μL of SYBR green PCR master mix (Quintect; Qiagen), 4 μL of water, and 1 μL (final concentration 0.5 μM) each of forward (gctgtggttgacgccggtatcagacac) and reverse (tttagttttagtgacgcatttgga) primers. The primers were designed to amplify a 125-bp portion of the conserved constant segment 3 of the *omp1* gene.

With each run of the assay a calibration curve was generated by the quantitation of a series of 10-fold dilutions of a standard of known concentration of *C. trachomatis* from 10^5 down to 1 copy per thermocycler capillary tube. *omp1* standards were produced by PCR amplification of purified *C. trachomatis* DNA using tube primers described, followed by gel purification of the product. The amount of product was estimated by a DNA assay (PicoGreen; Molecular Probes, Cambridge Biosciences, Cambridge, UK) using a microplate fluorometer (SpectraMax; Molecular Devices, Sunnyvale, CA). The number of copies of *omp1* product was then estimated using the calculated concentration, Avogadro’s number, fragment size, and the molecular mass of a single base pair in Daltons. Serial dilutions of the *omp1* product were made in ultrapure autoclaved water containing a constant amount of neutral DNA (Herring Sperm DNA; Sigma-Aldrich, Poole, UK) at 2 ng/μL. Standards were aliquoted and stored at −20°C. A PCR product was obtained in 46 (65%) of 71 reactions containing the one-copy standard. Because of sampling variability at very low concentrations, one would expect only 63% of these capillary tubes to contain one copy of the standard. As the observed and predicted rates of successful PCR amplification at the one-copy level are very similar, it is reasonable to conclude that the estimation of the standard concentration is accurate and that this assay works reliably down to the one-copy level. Precautions were taken to avoid contamination; sample processing, preparation of PCR mixtures, and post-PCR testing were all performed in separate rooms.

**Data Analysis**

Multivariable logistic regression models for infection and disease (as binary outcome variables) were developed on computer, using generalized estimating equations (GEE), taking account of clustering of disease and infection (Stata, version 7; Stata Corp., College Station, TX). Clustering was found at different levels (within village, compound, or room). Multilevel modeling (MLwiN, version 1.02; Institute of Education, London, UK) indicated that the levels of clustering were themselves associated, and so it was not possible to differentiate their effects reliably. Therefore, only the intermediate clustering level, compound, was used for the main GEE analysis.

The quantitated load of infection was expressed as the number of copies of *omp1/swab* and is the geometric mean of the two replicate assays. The Amplipicr assay is more sensitive than the quantitative assay, because for each copy of *omp1* there are multiple copies of the plasmid and, in addition, the volume of initial DNA extract used in the PCR reaction is greater for the Amplipicr test. Assuming there are 4 copies of the plasmidial plasmid for every 1 copy of the chlamydial chromosome, only 63% of these capillary tubes to contain one copy of the standard. As the observed and predicted rates of successful PCR amplification at the one-copy level are very similar, it is reasonable to conclude that the estimation of the standard concentration is accurate and that this assay works reliably down to the one-copy level. Precautions were taken to avoid contamination; sample processing, preparation of PCR mixtures, and post-PCR testing were all performed in separate rooms.
regression. Both component models were fitted simultaneously by maximum likelihood. The age-specific mean was estimated as the fitted probability of being positive, multiplied by the fitted mean among the positives. To assess whether the fitted mean varied with age, the means at 2.5 years (maximum) and 80 years were compared on computer using 1000 bootstrap replicates (S-Plus, ver. 6; Insightful Corp. UK, Bagshot, UK). The intraclass correlation estimate is based on a comparison of the within- and between-group sums of squares from an analysis of variance (ANOVA).

**Ethical Permission**

The design and procedures of this study were approved by the Gambian Government/Medical Research Council Joint Ethics Committee (Scientific Co-ordinating Committee Number 856) and the London School of Hygiene and Tropical Medicine Ethics Committee and adhere to the tenets of the Declaration of Helsinki. Informed consent from the head of each family was obtained before enrollment in the study.

**RESULTS**

**Study Population**

The study population lived in a cluster of 14 villages. Situated at the lower edge of the Sahel, the villages are typical of rural communities across the Sene-Gambian region. The total population of the study area was 1595. Village populations ranged from 23 to 321 (mean, 115). During the clinical survey in April 2001, 1519 (83%) people were examined and sampled. Most (88%) of those not examined had temporarily traveled. More than half of the population (54%) was under 16 years of age. The male-to-female ratio was equal in children less than 10 years of age; however, above this age, women outnumbered men, accounting for 62% of those aged 16 years or more. The study population lived in a cluster of 14 villages. Situated at the lower edge of the Sahel, the villages are typical of rural communities across the Sene-Gambian region. The total population of the study area was 1595. Village populations ranged from 23 to 321 (mean, 115). During the clinical survey in April 2001, 1519 (83%) people were examined and sampled. Most (88%) of those not examined had temporarily traveled. More than half of the population (54%) was under 16 years of age. The male-to-female ratio was equal in children less than 10 years of age; however, above this age, women outnumbered men, accounting for 62% of those aged 16 years or more. The population consists of the Wolof (55%) and Fula (45%) ethnic groups.

Farming is the main occupation, and a variety of livestock are reared. The animals are allowed to move freely around the compounds but are usually housed in pens overnight. All the villages have wells providing water throughout the year within a few minutes’ walk of each compound. Three percent of the population had attended a government primary school. Koranic education takes place in the village Madrassas, with 18% of adults reading some Arabic and 2% able to read English.

There was no organized trachoma control program in these villages before the survey. There is a local health center 7 km from the study area, which a few adults had attended for trichiasis surgery, and it is likely that children who were occasionally brought there with active trachoma would have received tetracycline ointment. A minority of families (43%) had constructed pit latrines.

**Active Disease and Infection**

Clinically active trachoma was diagnosed in 103 (7.7%) people of all ages (Fig. 1). Chlamydial DNA was detected by qualitative PCR in samples from 95 (7.2%) subjects. The clinical signs of active trachoma were associated with infection (odds ratio [OR], 4.5; \( P < 0.001 \); 95% confidence interval [CI], 2.7–7.7). However, many individuals had disease without detectable chlamydial DNA, and conversely there were many clinically normal individuals in whom *C. trachomatis* DNA was detected (Table 1). Severe inflammatory trachoma (grades P3 or TI) was more strongly associated with infection (OR, 12.7; 95% CI, 5.03–32.1) than was follicular trachoma (F2/3 or TF; OR, 3.18; 95% CI, 1.71–5.92).

**Quantitated Load of Chlamydial Infection**

*C. trachomatis* DNA was quantitated in 94 of the 95 samples positive by qualitative PCR. The distribution of the estimated number of copies of *omp1/swab* and by inference the number of *C. trachomatis* organisms was skewed with most infected individuals having relatively low copy numbers, although a few had very high copy numbers (Fig. 2). In addition, the distribution appeared to have a bimodal shape, with a low mode of 20 copies and a higher mode of 350 copies.

**Load of Infection and Clinical Phenotype**

The geometric mean of the estimated *omp1* copy number in clinically normal but infected individuals was 112 copies *omp1/swab* (95% CI, 66–189) whereas in those with signs of active disease it was 457 copies *omp1/swab* (95% CI, 92–2279). The medians and geometric means of the load of infection appeared to have a bimodal shape, with a low mode of 20 copies and a higher mode of 350 copies.

![Figure 1](http://www.jovs.org/pdfs/2003/44/10/4217.jpg)

**Figure 1.** The prevalence of clinically active disease and *C. trachomatis* infection (determined by qualitative PCR) by age.

![Figure 2](http://www.jovs.org/pdfs/2003/44/10/4217.jpg)

**Figure 2.** Distribution of the estimated quantitated load of infection (copies of *omp1/swab*), for samples found positive by qualitative PCR.

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**Table 1. Clinically Active Disease by Infection Status, as Determined by Qualitative PCR**

<table>
<thead>
<tr>
<th>Active Disease</th>
<th>+</th>
<th>–</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection status</td>
<td>+</td>
<td>25</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>80</td>
<td>1,144</td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
<td>1,216</td>
<td>1,319</td>
</tr>
</tbody>
</table>
significant increase in the mean infection load was found with increasing severity of both clinical signs, but was more marked with papillary inflammation. The heaviest infections were found in those with severe inflammatory trachoma (P3 or TI).

Load of Infection and Age

The prevalence of active disease was highest in children under 5 years (21.4%) and declined with increasing age (Fig. 1). In contrast, the prevalence of infection did not vary significantly with age. The modeled age-specific mean of infection load, taking into account the probability of being infected, did not vary significantly with age (Fig. 3). The mean difference between the maximum and minimum points of the modeled load by age was 53 copies of omp1/swab (95% CI, 19 to 196) on 1000 bootstrap replicates. However, partly because of the age distribution of the population, 69% of the quantitated chlamydial DNA was collected from children under 10 years.

Risk Factors for Low and High Loads of Infection

The relationship between the estimated infection load and various risk factors was investigated by separately comparing the individuals from the lowest third (low load, <34 copies of omp1/swab) and the highest third (high load, >390 copies of omp1/swab) of the quantitation distribution (Fig. 2) with all the noninfected individuals. Univariate analyses of these comparisons are presented in Table 3. The patterns of the age-specific prevalence of low- and high-load infection were different (Fig. 4). High-load infection peaked at 4.1% in the 5- to 9-year age group and declined to 0.9% in those more than 30 years of age. In contrast, the prevalence of low-load infection varied between 2.1% and 2.9% across the different age groups, with the exception of the 10- to 14-year-olds who had a prevalence of 1.0%. As water supply was equally good in all villages, water could not be assessed as a risk factor.

Multivariate logistic regression models for both low- and high-load infection were constructed adjusting for compound level clustering by GEE. Low-load infection was significantly associated with clinically active disease, no latrine access, sharing a bedroom with another person with high-load infection and living in a compound with a person who had clinically active disease (Table 4). High-load infection was associated with intense inflammatory trachoma (P3/TI), no latrine access,

TABLE 2. The Prevalence of Infection and the Median, Range, and Geometric Mean of the Estimated Load of Infection (Copies of omp1/swab) by Papillary and Follicular Grades in Those Individuals Positive by Qualitative PCR

<table>
<thead>
<tr>
<th>Qualitative PCR Positive (%)</th>
<th>Median Copies omp1/swab</th>
<th>Range Copies omp1/swab</th>
<th>Geometric Mean Copies omp1/swab*</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papillary grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P0</td>
<td>65/1,182 (5.5)</td>
<td>108</td>
<td>4.2-85,401</td>
<td>107</td>
</tr>
<tr>
<td>P1</td>
<td>10/69 (14.5)</td>
<td>360</td>
<td>4.2-4,495</td>
<td>137</td>
</tr>
<tr>
<td>P2</td>
<td>11/49 (22.5)</td>
<td>302</td>
<td>10.4-6,900</td>
<td>182</td>
</tr>
<tr>
<td>P3</td>
<td>9/19 (47.4)</td>
<td>1,961</td>
<td>30-797,401</td>
<td>5,523</td>
</tr>
<tr>
<td>Follicular grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F0</td>
<td>69/1,146 (6.02)</td>
<td>108</td>
<td>4.2-85,401</td>
<td>105</td>
</tr>
<tr>
<td>F1</td>
<td>4/74 (5.41)</td>
<td>1,522</td>
<td>4.29-4,495</td>
<td>1,794</td>
</tr>
<tr>
<td>F2</td>
<td>6/62 (9.68)</td>
<td>159</td>
<td>4.2-797,401</td>
<td>244</td>
</tr>
<tr>
<td>F3</td>
<td>16/57 (43.24)</td>
<td>350</td>
<td>10-660,421</td>
<td>707</td>
</tr>
</tbody>
</table>

* Test for trend (Cuzak-Wilcoxon rank sum test). Geometric mean copies of omp1/swab: papillary grade, z = 2.73; P < 0.01; follicular grade z = 1.99; P < 0.05.
Identifying Those Needing Antibiotics for Trachoma

and sharing a bedroom with someone with active disease (Table 5). Other factors (detailed in Table 3) were not significantly associated with either of these infection states. Interaction terms were assessed, including those between age, sex, and disease. None was statistically significant in models for infection.

**Infection without Clinical Signs of Active Disease**

Overall, clinically normal individuals in whom chlamydial DNA was detected tended to have lower infection loads than those with active disease, as detailed earlier. However, 23 of 32 of those from the highest third of the quantitation distribution (\(>390\) copies of \(omp1/swab\)) were clinically normal. The clinically normal but infected group were compared with all uninfected clinically normal individuals, to identify factors that might distinguish them. These groups were comparable in age, sex, and ethnicity. However, no latrine access and living in a compound with individuals having clinically active trachoma were significant risk factors for infection among clinically normal people (Table 6).

**Clustering of Disease and Infection**

Active disease and infection clustered significantly at the bedroom, compound, and village levels. Within the study area wide variations in their prevalence were found between villages only a few hundred meters apart (Fig. 5). In some villages neither infection nor disease was present, whereas in several other villages, little or no infection was detected, despite the presence of clinically active trachoma. Most of the infected individuals lived in three villages (1, 5, and 14). The intraclass correlation coefficient for village is 0.21 (95% CI, 0.21–0.22).

**Table 4.** A Multivariate Logistic Regression Model for Individuals with Infection Loads from the Lowest Third of the Quantitation Distribution (<34 copies of \(omp1/swab\)) Compared with Noninfected Individuals

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>P</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active disease (TF or TI)</td>
<td>2.62</td>
<td>0.002</td>
<td>1.44–4.74</td>
</tr>
<tr>
<td>No latrine access</td>
<td>5.67</td>
<td>0.030</td>
<td>1.18–27.1</td>
</tr>
<tr>
<td>Person with high-load infection in room</td>
<td>7.33</td>
<td>&lt;0.001</td>
<td>3.34–16.1</td>
</tr>
<tr>
<td>Other case of active trachoma in compound</td>
<td>10.80</td>
<td>0.023</td>
<td>1.39–83.2</td>
</tr>
</tbody>
</table>

Data are adjusted for compound level clustering by GEE.

**Figure 4.** Prevalence of low-level (<34 copies of \(omp1/swab\)) and high-level (>390 copies of \(omp1/swab\)) infection by age.
TABLE 5. A Multivariate Logistic Regression Model for Individuals with Infection Loads from the Highest Third of the Quantitation Distribution (≥390 copies of omp1/swab) Compared with Noninfected Individuals

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>P</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intense trachoma (P3 or TI)</td>
<td>17.7</td>
<td>&lt;0.001</td>
<td>6.08–51.6</td>
</tr>
<tr>
<td>No latrine access</td>
<td>12.9</td>
<td>0.017</td>
<td>1.58–105</td>
</tr>
<tr>
<td>Other case of active trachoma in room</td>
<td>4.06</td>
<td>&lt;0.001</td>
<td>2.18–7.47</td>
</tr>
</tbody>
</table>

Data are adjusted for compound level clustering by GEE.

for compound is 0.28 (95% CI, 0.26–0.30) and for room 0.27 (95% CI, 0.18–0.36).

Effectiveness of Different Treatment Strategies

The effectiveness of different antibiotic distribution strategies was modeled for the study area. Treatment coverage of infected individuals and those with more than 100 copies of omp1/swab that would have been achieved with different approaches were estimated (Table 7). If treatment were given only to those with signs of active disease, only one quarter of the infected individuals would have received antibiotic. If antibiotic distribution were extended to include all those who share a bedroom with someone with signs of active disease then approximately half the infected cases would receive treatment. If antibiotic were given to all the residents of a compound where one or more cases of active trachoma were found then 95% of infected individuals would receive treatment; however, the number of people treated per infected individual would be high (9.55). Alternatively, if treatment were given to all the residents of villages where the prevalence of active trachoma was 15% or greater in children 10 years of age and less, then almost 90% of infected individuals would be treated but with fewer people given treatment per infected case (5,59).

DISCUSSION

This study investigated the distribution and determinants of conjunctival load of C. trachomatis infection in a trachoma-endemic community in a rural area of The Gambia. To this end, a quantitative PCR methodology was applied to determine the number of copies of the chlamydial omp1 gene in conjunctival swab samples. Clinically active trachoma was significantly associated with the presence of C. trachomatis infection. However, less than a quarter of clinically active cases had detectable infection and conversely less than a quarter of infected individuals had signs of active disease (Table 1). In part, this disparity could reflect different time courses of infection and disease episodes with the onset of infection preceding clinical signs and the resolution of the signs lagging that of infection.17 Similar findings were reported in an earlier study from this region, in which 59% of infected individuals were clinically normal.6 Previously, it has been unclear whether infected but clinically normal individuals represent a significant reservoir of C. trachomatis that could infect others if left untreated, or alternatively whether they generally have low-level conjunctival infection or contamination with a few organisms.

The distribution of the estimated load of chlamydial infection was highly skewed and possibly bimodal in shape, suggesting the presence of two distinct groups of infected individuals (Fig. 2). The first group, with the higher mode, could arise from those with a productive infection, whereas in the second group, the lower omp1 copy numbers may reflect a transient inoculation with a subinfectious dose of the organism or individuals clearing the infection. However, the cross-sectional design of this study does not allow us to distinguish between these possibilities.

Overall, individuals with active disease had higher chlamydial loads than those without. Severe inflammatory trachoma was strongly associated with higher loads of infection. However, most of those from the highest third of the quantitation distribution (≥390 copies of omp1/swab) were clinically normal. This may again be due to differences in the time course of infection and disease and the previously described observation that adults with conjunctival chlamydial infections have signs of active trachoma for only a short period.17 A significant increase in infection load was found with increasing papillary inflammation and a less marked increase with increasing follicular score (Table 2). The weaker association with follicular response suggests that follicles may reflect a more effective immune response to C. trachomatis or that they develop later in an infection episode, after the chlamydial load has begun to decline. This is consistent with a study from Tanzania, in which a ligase chain reaction for plasmid DNA was used in a semi-quantitative way to assess chlamydial infection loads. Severe inflammatory trachoma was associated with persistent C. trachomatis and higher loads of infection.18

The interaction between trachoma and age is complex in this environment. Although the signs of active disease declined with increasing age, neither the prevalence of infection nor the mean infection load declined (Fig. 3). Previous studies have found that the duration of disease and infection episodes declines with age.17 However, in this low-prevalence setting infrequent exposure to C. trachomatis may limit the development and maintenance of protective immune responses, resulting in heavier infections when they occur. This observation may have implications for the design of trachoma control programs in low-prevalence regions, as failing to treat older infected individuals may leave a significant reservoir of infection within a community.

TABLE 6. A Multivariate Logistic Regression Model for the Presence of Infection in Clinically Normal Individuals

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>P</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No latrine access</td>
<td>9.77</td>
<td>&lt;0.001</td>
<td>3.13–30.5</td>
</tr>
<tr>
<td>Case of active trachoma in compound</td>
<td>11.2</td>
<td>0.005</td>
<td>2.08–59.9</td>
</tr>
</tbody>
</table>

Data are adjusted for compound level clustering by GEE.

FIGURE 5. Prevalence of clinically active disease in children 10 years and under and the prevalence of C. trachomatis infection by qualitative PCR for all ages, by village.
The presence of a latrine in the family compound appeared to have a protective effect. This finding is consistent with research conducted in this area of The Gambia, which has demonstrated that the eye-seeking fly *Musca sorbens* breeds preferentially in human fecal deposits on the ground and, in this environment, is responsible for 90% of fly–eye contacts. In a pilot intervention study, fly control with insecticide spray was associated with a significant reduction in the number of new cases of trachoma over a 3-month period. This led to the suggestion that pit latrines may reduce the transmission of trachoma. Clustering of both disease and infection was prominent within the study area and occurred at the village, compound, and bedroom levels. Similar spatial clustering of trachoma has been described in a number of other studies and is evidence for the importance of intrafamily transmission of infection. Sharing a bedroom appears to be an important risk factor for trachoma transmission, probably reflecting living in close proximity to each other throughout the day as well as at night. High-load infection was associated with sharing a bedroom with a person with clinically active trachoma, while sharing a bedroom with a person with high-load infection was a risk factor for having low-level infection. These associations did not lose significance when the number of people sharing a room was considered in the analysis, suggesting that they are not simply markers for crowded living conditions. The variation in the relative prevalence of disease and infection between villages may reflect their dynamic interplay in a relatively low-prevalence setting (Fig. 5). The infrequent introduction of *C. trachomatis* into a community permits the resolution of the infection and subsequently the associated clinical signs before the next episode occurs. In a study from a low-prevalence region of Nepal (6% active disease in children), no child with signs of active trachoma was found to be infected with *C. trachomatis*. This could be a more extreme example of the loose relationship between signs and infection that was observed in the present study and may be a feature common to low-prevalence settings.

The present study highlights two particular challenges faced by trachoma control programs in low-prevalence settings in identifying infected individuals in need of treatment: first, the mismatch between clinical signs and infection and, second, the marked clustering of disease. A strategy that targets treatment only to individuals with signs of active trachoma would miss most of the cases of infection in this community (Table 7). However, as most of the infection occurs in clusters, which usually contain one or more individuals with signs of active trachoma, a strategy that involves treating a whole unit of people containing one or more cases of clinically active disease would be more likely to succeed in delivering treatment to infected individuals. An empirical comparison of the potential effectiveness of different approaches demonstrated that, in this study area, mass antibiotic treatment of an entire village where the prevalence of active disease was 15% or more in children would deliver treatment to 90% of infected cases and would be a relatively efficient use of resources. However, the prevalence level of active disease that indicates that mass treatment would be worthwhile will probably vary in different settings. The conjunctival load of *C. trachomatis* is likely to be determined by a complex interaction of host, organism, and environmental factors. The size and frequency of inocula will influence whether an infection becomes established and will largely depend on the prevalence of infection in a community and the case with which transmission events occur. There is a need to investigate the interaction between infection, immunologic response, clinical manifestations, and the scarring process at the conjunctival surface. Such studies would provide valuable insights into human chlamydial infections in general. Some caution is needed in interpreting the quantitation results. Although swabs were taken by a standardized method, it is clear that some individuals would comply with the procedure better than others. More cellular material is likely to be collected from subjects with severe inflammation. Thus, it is unlikely that similar amounts of human conjunctival cells are collected from each individual. The ratio of chlamydial DNA to that of a human housekeeping gene may give a more accurate indication of the variability in infectious load. However, it is also possible that additional free chlamydial elementary bodies were collected from the tear film. At the cellular level, the relationship between human and chlamydial signals may be expected to vary with the stage of the chlamydial life cycle, which would further complicate the measurement of the load of infection. However, despite these limitations, the amount of chlamydial DNA collected by the swab is likely to reflect the relative importance of an individual as a source of infection to others, because what the swab collects is probably proportionate to what would be collected by fomites and other modes of transmission.

### Table 7. The Effectiveness of Different Antibiotic Distribution Strategies in Delivering Treatment to All Individuals Infected with *C. trachomatis* and for Those Individuals in Whom More Than 100 Copies of omp1/swab Were Detected

<table>
<thead>
<tr>
<th>Treatment Distribution Strategy</th>
<th>Total Number of People Treated (n = 1319)</th>
<th>All Infected Individuals</th>
<th>Infected Individuals with &gt;100 Copies omp1/swab</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only active cases</td>
<td>103</td>
<td>23 (24.2%)</td>
<td>4.48</td>
</tr>
<tr>
<td>All people who share a room with</td>
<td>305</td>
<td>46 (48.4%)</td>
<td>6.63</td>
</tr>
<tr>
<td>clinically active disease</td>
<td>1+ case</td>
<td>91 (95.7%)</td>
<td>9.55</td>
</tr>
<tr>
<td></td>
<td>2+ cases</td>
<td>76 (80.0%)</td>
<td>7.87</td>
</tr>
<tr>
<td>All residents of a compound where</td>
<td>869</td>
<td>91 (95.7%)</td>
<td>9.55</td>
</tr>
<tr>
<td>active disease was found</td>
<td>458</td>
<td>85 (89.5%)</td>
<td>9.35</td>
</tr>
<tr>
<td>All residents of a village with 15%+ clinically active disease in children</td>
<td>458</td>
<td>85 (89.5%)</td>
<td>3.59</td>
</tr>
<tr>
<td>All children of a village with 15%+ clinically active disease in children</td>
<td>458</td>
<td>85 (89.5%)</td>
<td>9.35</td>
</tr>
<tr>
<td>All residents of study villages 1, 3, and 14</td>
<td>261</td>
<td>54 (56.8%)</td>
<td>4.83</td>
</tr>
<tr>
<td></td>
<td>316</td>
<td>85 (87.4%)</td>
<td>3.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>48 (87.2%)</td>
</tr>
</tbody>
</table>

...
In conclusion, in this study, we measured the community distribution of the load of *C. trachomatis* infection in a trachoma-endemic area of The Gambia. Most infected individuals did not have signs of active disease. However, overall, these cases tended to have lower chlamydial infection loads when compared with infected individuals with signs of active trachoma. High loads of infection were strongly associated with severe inflammatory trachoma and occurred more commonly in younger age groups, although the mean load of infection varied little with age. Infection and disease clustered, emphasizing the importance of intrafamily transmission and were associated with absence of latrines, suggesting that improved sanitation may be a useful adjunct to antibiotic therapy. These data suggest that in areas of low endemicity, such as The Gambia, antibiotic distribution strategies should include the treatment of clinically normal people who live alongside individuals with clinically active trachoma to maximize the delivery of treatment to those infected with *C. trachomatis*.

Acknowledgments

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References