Strategies for control of trachoma: observational study with quantitative PCR


Summary

Background Antibiotics are an important part of WHO’s strategy to eliminate trachoma as a blinding disease by 2020. At present, who needs to be treated is unclear. We aimed to establish the burden of ocular Chlamydia trachomatis in three trachoma-endemic communities in Tanzania and The Gambia with real-time quantitative PCR.

Methods Conjunctival swabs were obtained at examination from 3146 individuals. Swabs were first tested by the qualitative Amplicor PCR, which is known to be highly sensitive. In positive samples, the number of copies of omp1 (a single-copy C trachomatis gene) was measured by quantitative PCR.

Findings Children had the highest ocular loads of C trachomatis, although the amount of pooling in young age groups was less striking at the site with the lowest trachoma frequency. Individuals with intense inflammatory trachoma had higher loads than did those with other conjunctival signs. At the site with the highest prevalence of trachoma, 48 of 93 (52%) individuals with conjunctival scarring but no sign of active disease were positive for ocular chlamydiae.

Interpretation Children younger than 10 years old, and those with intense inflammatory trachoma, probably represent the major source of ocular C trachomatis infection in endemic communities. Success of antibiotic distribution programmes could depend on these groups receiving effective treatment.

Lancet 2003; 362: 198–204
See Commentary

Introduction

Trachoma accounts for 10–15% of global blindness.1 It is caused by ocular infection with Chlamydia trachomatis and mainly affects poor, marginalised, and displaced people living in the hot dry regions of the world. Blindness from trachoma is preventable. In 1998, WHO set a target of global elimination of trachoma as a blinding disease by the year 2020.2 The SAFE strategy—surgery for in-turned eyelashes, antibiotics for active disease, face washing (or promotion of facial cleanliness), and environmental improvement to reduce transmission—is to be used in pursuit of this objective.

The antibiotic of first choice against trachoma is now azithromycin: it is effective,3–7 well tolerated,3,4–8 and—because one oral dose produces high cure rates—is simple to administer.8 Yearly mass treatment of communities in which trachoma is hyperendemic has therefore been recommended.6,10 However, azithromycin is expensive if it is not donated,11 and mass distribution of the drug could lead to development of macrolide resistance in C trachomatis or other human pathogens.12–14 Depending on the level of endemicity, researchers have suggested that treatment be given only to children younger than 10 years old,15 to children with active trachoma and their families,15 or to children with active trachoma and household contacts who are children.16 Because data showing the effect of these targeted antibiotic strategies are limited,15 establishment of the population subsets that harbour the greatest load of organism would be helpful to predict the approaches that will probably be most effective and cost effective.

Clinically, the highest prevalence of active disease is noted in young children. However, with the ligase chain reaction, Schachter and colleagues17 reported that 27% of individuals without signs of active trachoma were positive for C trachomatis DNA in Egypt and The Gambia, including a high proportion of adults. In a study in Tanzania, 24% of individuals without signs of active trachoma were reported to be positive for C trachomatis by PCR.18 We do not yet know whether such clinically negative individuals who are positive for C trachomatis harbour infection that is of epidemiological importance. If so, restriction of antibiotic treatment to individuals with clinical signs would have limited benefit for trachoma control. Alternatively, the data might just show that the high sensitivity of DNA amplification assays leads to positive results from individuals with transient self-limiting infections. Such people might be unlikely to act as a source for transmission of infection. Quantification of ocular chlamydial infection could help to answer this question.

The aim of our study was to measure—with real-time quantitative PCR—the amount of C trachomatis DNA present in conjunctival swabs taken from all consenting members of three separate trachoma endemic communities in Tanzania and The Gambia, and to
examine the relation between the quantitative burden of infection and age, sex, and clinical trachoma status. We used isolation in tissue culture to validate the relevance of the quantitative PCR results.

**Methods**

Research was done in accordance with the declaration of Helsinki. Ethics approval was obtained from the ethics committee of the London School of Hygiene and Tropical Medicine, UK, the research and ethical clearance committee of the Kilimanjaro Christian Medical Centre, Tanzania, the joint committee on clinical investigation at Johns Hopkins School of Medicine, USA, the National Medical Research Council in Tanzania, and the Gambian Government/Medical Research Council joint ethics committee. We obtained the support of local elders in every community before research began.

**Study population**

We undertook investigations in three communities, which we selected on the basis of trachoma prevalence, village size, and absence of previous involvement in formal trachoma control programmes. Kahe Mpya sub-village of Kahe village, Rombo District, Tanzania, is on the northeastern slopes of Mount Kilimanjaro, placing it in the rain shadow of the mountain. Maindi sub-village of Kongwa district is located on the Masai Steppe in a dry area of central Tanzania. The Jareng cluster of 14 villages lies in the Upper Saloum District, Central River Division, The Gambia. All are rural communities in which farming is the main occupation. We enumerated the population of the three communities. In Kongwa, all residents older than age 6 months were invited to participate in the study. In Rombo and Jareng, residents of all ages were eligible. We obtained written informed consent from all individuals, and from the parents of children deemed too young to give consent themselves.

**Procedures**

We examined the right eye (Rombo and Kongwa) or left eye (Jareng) of consenting individuals for signs of trachoma according to the 1981 modified WHO system (Jareng) or the 1987 WHO simplified system (Rombo and Kongwa); we standardised data from Jareng by changing the grades from the 1981 system to the equivalent ones from the 1987 system, for reasons of data analysis. In the WHO simplified system, trachomatous inflammation–follicular (TF) is the presence of five or more follicles in the central part of the upper tarsal conjunctiva; trachomatous inflammation–intense (TI) is pronounced inflammatory thickening of the upper tarsal conjunctiva that obscures more than half the normal deep tarsal vessels; and trachomatous conjunctival scarring (TS) denotes the presence of easily visible scarring in the tarsal conjunctiva.

After examination, a sterile dacron polyester-tipped swab (Puritan, Hardwood Products, Guilford, USA) was held horizontally and passed across the length of the upper tarsal conjunctiva four times, rotating it a quarter turn with every pass. In Jareng, we instilled one drop of proxymetacaine hydrochloride 0-5% topical anaesthetic (Minims, Chauvin Pharmaceuticals, Romford, UK) into the eye before swabbing. We did not use anaesthetic at either of the Tanzanian sites. We took precautions to prevent carry-over contamination of swabs taken from successive individuals, including ensuring that the swab head did not contact anything other than the person’s conjunctiva, not using scissors or blades to cut the swab shaft, and cleaning examiners’ hands between successive participants. Swabs were placed dry into 100% polypropylene tubes, kept on ice (4°C), then frozen at −20°C or lower before laboratory processing.

In Rombo, individuals with clinical evidence of active disease (TF, TI, or both) in the right eye had a second swab taken from the upper tarsal conjunctiva of the right eye. This swab was placed in sucrose phosphate containing 10% fetal calf serum, vancomycin, and gentamycin (2-SP medium), kept on dry ice (−78°C) until it was stored at −70°C (within 36 h of collection), and shipped on dry ice to Winnipeg, Canada, for isolation of C trachomatis.

**PCR**

For the dry swabs taken at all three sites, we used the Amplicor C trachomatis qualitative PCR assay (Roche Molecular Systems, Branchburg, NJ, USA) for the first stage of sample analysis, to select samples for quantification. Standard precautions against cross-contamination were used. Every swab was eluted by vortexing for 10 s in 0.5 mL Amplicor CT/NG (C trachomatis/Neisseria gonorrhoeae) lysis buffer in a 2 mL polypropylene tube. Excess liquid was expressed from the swab against the inside of the tube, and 0.3 mL Amplicor specimen diluent added. The lysed sample was held at room temperature for 10 min, incubated at 95°C for 10 min to inactivate inhibitors, and refrigerated overnight at 4°C.

We did PCR according to the manufacturer’s instructions, as described elsewhere.

We tested separately for amplification of both target plasmid DNA and the master-mix internal control sequence, to identify samples in which PCR was inhibited. Such samples were retested by a one in five dilution with a 50/50 mixture of Amplicor CT/NG lysis buffer and specimen diluent. The Amplicor test targets DNA of the common cryptic plasmid of C trachomatis. From every positive (optical density [OD] ≥0.8) and equivocal (0.2<OD<0.8 on two replicate tests) sample, 360 μL of Amplicor preparation was processed through a QIAamp DNA mini kit (Qiagen, Crawley, UK) to further purify and concentrate sample DNA, because Amplicor buffer is not compatible with the quantitative PCR assay.

We did real-time quantitative PCR with the LightCycler (Roche Molecular Systems). We added 4 μL of resuspended DNA from the QIAamp column to a glass capillary containing 16 μL prepared PCR master-mix. For all but 17 of the samples, quantification was done twice, on two 4 μL replicate samples in two independent quantitative PCR assays. The target for quantitative PCR amplification was a 123 bp fragment within the highly conserved constant domain 3 of the omp1 gene, located on the chromosome of C trachomatis. Every C trachomatis elementary body contains one copy of this gene. Primer sequences are shown in the panel.

To prepare standards for quantification, the target sequence was amplified and gel-purified. We accurately estimated the concentration of the stock with Picogreen (Molecular Probes, Eugene, OR, USA), which was read at 502/523 nm in the Molecular Devices SpectraMax and

**Primer sequences used in quantitative PCR method**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tr>
<td>CT1</td>
<td>5’-GCTGTTGTTGAGCCTTACACAC-3’</td>
</tr>
<tr>
<td>CT2</td>
<td>5’-TTTAGTTTAGATTGACATTTGGA-3’</td>
</tr>
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</table>
analysed with SoftMax Pro. Absolute copy number of the stock was estimated according to the formula:

\[
\text{number of molecules/\mu L} = \frac{\text{[g/\mu L] \times [fragment size (123 bp) \times 6 \times 0.022 \times 10^{11}]]}{\text{[fragment concentration} \times \text{[fragment size (123 bp)]}]}\]

Stock was serially diluted in sterile water containing 2 mg/L Herring sperm DNA (Sigma, Poole, Dorset, UK) so that 4 \mu L contained the required copy number of standard. Every assay included six concentrations of standard (ten-fold dilutions of the stock solution from 10° to one copy per capillary) and a negative or zero control (DNA-free and RNA-free water used in place of sample); these were used to generate a standard curve. Of 71 capillaries taken from the most dilute standard (one copy per capillary), 46 (65%) amplified successfully. 37% (=e–1) of such capillaries would be expected to contain no copies, simply because of sampling variability. This value is very close to the observed proportion (35%) of non-amplifying capillaries. Therefore, we concluded that, with this assay, we could reliably amplify one copy of the 123 bp fragment from omp1 per capillary. However, sampling variability also means, of course, that swabs of very low concentration could fail to test positive simply because their capillaries do not, by chance, contain any copies. Quantitative PCR on samples from all three sites was done with identical methods, on the same LightCycler at the London School of Hygiene and Tropical Medicine, UK, by the same laboratory personnel.

Culture

We inoculated eye swabs obtained in 2-SP medium into HeLa 229 cells in 12-well tissue culture plates with a previously described method with minor adaptations.23 We pretreated confluent 24-h old cell monolayers for 20 min with DEAE-dextran (30 mg/mL). Every swab was vortexed for 15 s with sterile glass beads and inoculated onto the pretreated HeLa cell monolayers in triplicate. We incubated the inoculated cultures at 35°C in 5% CO₂ for 1 h. Isolation media, containing minimal essential media, 10% fetal calf serum, 100 mmol/L glutamine, and 1 mg/L cycloheximide, were added to every well and the cultures were centrifuged at 1240 g for 60 min at 25°C. An additional 1 mL of isolation media was added to every well after centrifugation and the cultures were incubated at 35°C in 5% CO₂ for 72 h. One monolayer culture from every specimen was stained for the presence of chlamydial inclusions with a fluorescein-conjugated monoclonal antibody specific for C trachomatis (C trachomatis culture confirmation kit, Syva, Palo Alto, USA). The remaining two monolayers were passaged in a masked approach onto fresh HeLa cell monolayers. A specimen was deemed negative if no inclusions were detected after ten masked passages.

Statistical analysis

Results from quantification have been shown as the estimated number of copies of omp1 per swab. We obtained these numbers by multiplication of the copy number per capillary measured by quantitative PCR, taking into account the volumes used in every processing step. Such manipulation assumes that all DNA gathered onto the swab passed into the Amplicor lysis buffer in the first stage of sample processing.

Samples that were positive on Amplicor but which did not generate specific product by quantitative PCR were deemed positive. Amplicor should be more sensitive than our quantitative assay, for two reasons. First, the 4 \mu L of purified and resuspended DNA used for quantification is equivalent to 28.8 \mu L of the original Amplicor preparation; 50 \mu L of that preparation is used in the Amplicor assay. Second, Amplicor detects a multi-copy plasmid rather than a single-copy chromosomal gene, multiplying the volume advantage. Amplicor-positive samples could therefore test negative by quantitative PCR simply because of sampling variability, despite the fact that the quantitative assay was enhanced so it could amplify one copy of omp1 per capillary. On this basis, we obtained a probable estimate of the density of the Amplicor positive, quantitative PCR-negative swabs (see webappendix: http://image.thelancet.com/extras/02art11304webappendix.pdf). On the assumption of four plasmids per organism,14 and in view of the sample volumes used, the maximum likelihood estimate is

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>Rombo</th>
<th>Kongwa</th>
<th>Jareng</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–15</td>
<td>529/534 (99%)</td>
<td>461/532 (87%)</td>
<td>762/874 (87%)</td>
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<tr>
<td></td>
<td>16–30</td>
<td>152/161 (94%)</td>
<td>230/283 (81%)</td>
<td>261/359 (73%)</td>
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<tr>
<td></td>
<td>&gt;30</td>
<td>275/283 (97%)</td>
<td>180/202 (89%)</td>
<td>297/381 (78%)</td>
</tr>
</tbody>
</table>

Data are number tested/total (%).
village—a frequent occurrence, particularly in the dry season, when many people leave their home village for weeks at a time. Nine individuals in Jareng refused to take part. Clinical information and Amplicor results are available for 1319 (83%).

The point prevalences of active disease (all ages) were 174/956 (18%), 312/871 (36%), and 103/1319 (8%) in Rombo, Kongwa, and Jareng, respectively. Age-standardised prevalences were 19%, 37%, and 8%, respectively. Figure 1 shows the prevalence of active disease by age group for every site. In every age group, the highest prevalence of active disease was found in Kongwa, and the lowest in Jareng.

In Rombo, Kongwa, and Jareng, 91/956 (10%), 496/871 (57%), and 95/1320 (7%), respectively, tested for 5.7 organisms per swab (95% CI 0.27–44.9). Most samples had two replicates. For those positive on Amplicor but with two negative quantitative results, we estimated the number per sample by maximum likelihood as 4.1 (0.23–24.9). For other samples, the geometric mean of the two replicates is presented here (including some with the value of 5.7 inferred for one of the replicates). Of all samples tested by quantitative PCR, 17 (15 from Rombo, one from Kongwa, and one from Jareng) could only be tested once, because of low volumes of residual sample after optimisation of the assay.

We double-entered data in Microsoft Access (version 97 SR-2) in Rombo, and checked discrepancies against the original forms. In Kongwa and Jareng, data were single-entered in Microsoft Access, and every entry was checked by hand by the data manager. We did the statistical analysis with Stata 7 (Stata, College Station, TX, USA) and S-PLUS 6.0 (Insightful, Seattle, WA, USA). Age standardisation was done with the direct method, and we used the three-site total as the standard population. We compared prevalences between groups by the \( \chi^2 \) test, and densities by Kruskal-Wallis. In Kongwa and Jareng, we rounded down ages to the nearest year. When age was analysed as a continuous variable, 0.5 was added to the ages from those sites, to give the mid-year estimate.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Of 978 residents of Kahe Mpya in Rombo, 956 (98%) were examined and swabbed (table 1), two were temporarily absent from the village, five explicitly refused, and 15 did not attend for examination. In Kongwa, 874 of 1017 residents (86%) were examined and swabbed, 130 were temporarily absent from the village for agriculture, cattle herding, or other reasons, one refused, and 12 were ineligible because they were younger than 6 months of age. Of the 874, no clinical data were recorded for one individual, and swabs were unavailable for two others: clinical information and Amplicor results were available for 871. In Jareng, 1342 of 1595 (84%) residents presented; 22 of 1342 were examined but not swabbed, and one was swabbed but not examined. Most of those not seen (244/1595, 15%) had travelled out of the village—a frequent occurrence, particularly in the dry season, when many people leave their home village for weeks at a time. Nine individuals in Jareng refused to take part. Clinical information and Amplicor results are available for 1319 (83%).

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In Rombo, Kongwa, and Jareng, 91/956 (10%), 496/871 (57%), and 95/1320 (7%), respectively, tested positive on Amplicor; Table 2 shows the number of copies of \( \text{omp1} \) per swab (geometric mean [95% CI]) for those positive on Amplicor at each passage. The geometric mean number of copies of \( \text{omp1} \) per swab was 5.7 (95% CI 0.27–44.9) for individuals with positive Amplicor results; for those positive on Amplicor but with two negative quantitative results, we estimated the number per sample by maximum likelihood as 4.1 (0.23–24.9). For other samples, the geometric mean of the two replicates is presented here (including some with the value of 5.7 inferred for one of the replicates). Of all samples tested by quantitative PCR, 17 (15 from Rombo, one from Kongwa, and one from Jareng) could only be tested once, because of low volumes of residual sample after optimisation of the assay.

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positive by Amplicor for *C trachomatis* plasmid DNA. Age-standardised prevalences were 10%, 57%, and 7%, respectively. A breakdown of these results by age group for every site is shown in figure 2.

The prevalence of infection was generally very similar in males and females at every site, and in the under-15 (0–15) and over-15 (>15) years age groups. The exception was under-15s in Kongwa, in whom females had 70% prevalence and males 59% (p=0.01). The number of copies of *omp1* recorded in ocular swabs did not differ between the sexes, at any site.

From Rombo, right-eye swabs for culture were available for 56 of 58 individuals with active disease in the right eye who were reported to be Amplicor-positive in that eye. Of this group, only those individuals who were also positive for quantitative PCR were culture-positive; all five individuals for whom both quantitative PCR replicates were negative were also culture-negative (table 2).

Figure 3 shows scatter plots of the estimated number of copies of *omp1* per swab by age and sex for every site. Only data points for samples positive by Amplicor and tested by quantitative PCR have been included on these plots. After matching of purified DNA to the original sample codes, of 496 Amplicor-positive samples from Kongwa, only 412 were tested by quantitative PCR. Similarly, one sample (of 95 positives) from Jareng could not be quantified. At the Tanzanian sites (Rombo and Kongwa), most individuals with high copy numbers per swab were young, and only a small proportion of adults were in this category. In Jareng, individuals with high copy numbers were more diffusely distributed by age than in Tanzania. The difference is shown by considering the cumulative load of *C trachomatis* with increasing age: in Rombo, 50% of the total community swab-load of *C trachomatis* DNA was recorded in samples from children age 9 months old and younger, even though children in this age bracket contributed only five of the 91 samples positive for *C trachomatis*. More than 90% of the Rombo community load was recorded in samples from children aged 6–6 years or younger. These age brackets for fractions-of-total-load are heavily affected by two female infants aged 5 months and 8 months, whose swabs together contained nearly 60% of the total community burden of ocular *C trachomatis*. If these two children were excluded from the analysis, 50% of the remaining load was noted in residents younger than 4 years and 2 months, and 90% in those aged younger than 9 years and 6 months; nearly 12% of all ocular *C trachomatis* is still recorded in those younger than 1 year old. Corresponding upper age markers for 50% and 90% of total burden were 3–5 and 6–5 years for Kongwa, and 5–5 and 27–5 years for Jareng.

Frequency distributions for the number of individuals at every site having various *C trachomatis* loads are shown in figure 4. Amplicor-negative individuals (presumed to have zero load) have not been included. Every population had many individuals with a small number of copies of *omp1* in their conjunctival swabs, and a few individuals with very heavy infections. However, those positive for *C trachomatis* in Jareng had higher densities than did those positive at Rombo and Kongwa. The difference between Jareng and the other sites was significant for individuals older than 15 years of age (p<0.0001), but not for the 0–15 years age group (p=0.07), despite the higher power because of the large number of positive samples.

Table 3 shows the prevalence of infection in groups of individuals with different combinations of (swabbed eye) clinical signs, stratified by site. The geometric mean of the estimated number of copies of *omp1* in Amplicor-positive individuals is shown by clinical status in table 4. Substantially higher copy numbers were recorded in

<table>
<thead>
<tr>
<th></th>
<th>Rombo</th>
<th>Kongwa</th>
<th>Jareng</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF without TI</td>
<td>17/63 (27%)</td>
<td>161/217 (74%)</td>
<td>14/84 (17%)</td>
</tr>
<tr>
<td>TI with or without TF</td>
<td>41/111 (37%)</td>
<td>80/95 (84%)</td>
<td>9/19 (47%)</td>
</tr>
<tr>
<td>TS with neither</td>
<td>2/86 (2%)</td>
<td>48/93 (52%)</td>
<td>8/81 (10%)</td>
</tr>
<tr>
<td>TF nor TI</td>
<td>31/696 (4%)</td>
<td>207/486 (44%)</td>
<td>64/1135 (6%)</td>
</tr>
<tr>
<td>No sign of trachoma</td>
<td>All individuals</td>
<td>91/956 (10%)</td>
<td>496/871 (57%)</td>
</tr>
</tbody>
</table>

Data are number of individuals who are Amplicor positive/total (%).
Amplicro-positive individuals who had TI than in those who had TF without TI, TS without any sign of active disease, or who had no sign of trachoma.

Discussion
We have shown the quantitative distribution of ocular C trachomatis infection in three whole trachoma-endemic communities. We recorded that—in two Tanzanian communities mesoendemic and hyperendemic for active trachoma—most of the C trachomatis organism was found in young children, whereas in a Gambian population with a low prevalence of active disease, the burden of infection was more evenly distributed in individuals of all ages. Our results have major importance for trachoma control programmes. Four findings in particular should be highlighted.

First, all data confirm the importance of children as the major reservoir of infection. In Rombo and Kongwa, more than 90% of the total quantity of chlamydial DNA recorded was from swabs taken from children younger than 9 years of age. Children younger than 10 years old therefore probably constitute the major source of organisms for transmission to others, and should be the main target group for antibiotic distribution programmes. Further, in Rombo, more than 50% of the total burden was in infants younger than 1 year of age; if two heavily infected infants are excluded from the analysis, children up to 1 year harbour nearly 12% of the remaining ocular load. This finding is important, because present Tanzanian guidelines exclude children younger than 1 year of age from receiving azithromycin as part of trachoma control campaigns. More data are needed on the safety of azithromycin for children in this age group.

Second, our data suggest that the age distribution of the load of ocular C trachomatis varies between sites of differing endemicity. High chlamydial loads were more diffusely distributed in different age groups in Jareng—which had the lowest prevalence of trachoma (TS) but no active disease were Amplicor positive in Kongwa. The chlamydial loads of these individuals were low (geometric mean number of copies of omp1 per swab was 24); whether such a load is important as a reservoir of infection will be established in our longitudinal studies. The presence of any amount of infection could, however, have implications for the individuals themselves: in a previous cohort study of women with conjunctival scarring in Kongwa, those who were positive for infection at 7 years’ follow-up (by PCR-directed at omp1), were significantly more likely to have developed trichiasis than were those negative for infection.25 In Jareng, individuals with TS but no active disease had a geometric mean number of copies of omp1 per swab of 298, which is higher than that index for individuals with TF alone at any of the three sites.

For those with clinical evidence of active disease, detection of chlamydial chromosomal DNA in a swab taken from the conjunctiva strongly correlated with the likelihood that the individual would be culture positive. Culture positivity indicates that viable chlamydiae are being shed, which suggests that the individual is able to infect contacts. The actual quantitative load that constitutes an important source of infection for others will need to be established by longitudinal studies. We are undertaking such studies in all three populations.

Every quantitative PCR assay used a 4 μL volume from a total of 50 μL QIAamp-eluted DNA. The LightCycler’s ability to work with small sample volumes in glass capillaries is one of its attractions, since this ability reduces the cost of reagents, and allows rapid thermal cycling and real-time detection of amplified product. However, small volumes also increase the effect of variability between samples and—as an extension of this phenomenon in our study—helped to increase the likelihood that samples with small amounts of chlamydial DNA would test positive by Amplicro but negative by quantitative PCR. We tried to compensate for sampling variability by doing two replicate assays per sample, and for the difference in results with the two PCR methods by estimating the copy number in Amplicro-positive, quantitative PCR-negative samples by maximum likelihood.

Contributors
All investigators contributed to the design and implementation of the study, and to writing or editing of the report. Principal investigators at study sites were A Solomon (Rombo), M Burton (Jareng), and S West (Kongwa); they, along with N Alexander and B Muñoz managed the study, and to writing or editing of the report. Principal investigators at study sites were A Solomon (Rombo), M Burton (Jareng), and S West (Kongwa); they, along with N Alexander and B Muñoz managed the data. A Solomon, M Burton, P Massue, H Mocha, and R Bailey undertook the fieldwork. M Holland and A Aguirre developed the quantitative PCR assay. R Peeling was responsible for chlamydial isolation in tissue culture. N Alexander undertook the analyses. D Mabey coordinated the project.
Conflict of interest statement
None declared.

Acknowledgments
We thank the village chairmen, elders, and villagers of Kabe, Maiindi, and Jareng for their advice, good humour, and participation; our field teams for help with collection of data and specimens, especially Nkoyo Faal, Angels Natividad-Sancho, Esther Aryee, and Shanshan Li for laboratory assistance; John Shao, Salesia Safari, and all the members of our research steering committees; and to the Wellcome Trust/Barroths Wellcome Fund, the Edna McConnell Clark Foundation, the International Trachoma Initiative, and the Alcon Institute for financial support.

References