Severe Disease in Children with Trachoma Is Associated with Persistent

*Chlamydia trachomatis* Infection

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The immediate study objective was to determine if variable disease severity in children with trachoma could be attributable in part to host variation in the ability to clear *Chlamydia trachomatis* infection. Identification of sibling cohorts with these variant phenotypes would be useful for immunogenetic studies. A weekly survey for 3 months in a trachoma-hyperendemic village using detection of chlamydial DNA and grading of disease severity indicated that 62% (35/56) of children had at least one infection episode. Of those, 64% (21/33) who were persistently infected had both significantly higher mean chlamydial DNA loads and more severe trachoma than did sporadically infected children. Of importance, duration of infection differed between siblings in 60% (6/10) of families. The results suggest that chlamydial load and duration of infection determine the chronic nature of severe disease in trachoma and that host variable efficiency for chlamydial clearance between siblings is in part determined by host variation.

*Chlamydia trachomatis*, the agent of trachoma, continues to be the leading infectious cause of blindness worldwide, with a predicted increase from 12 million to 30 million cases within the next 30 years [1]. Trachoma is hyperendemic in communities with limited economic resources where sanitation and hygiene are poor. In past studies in trachoma-endemic areas, >60% of children had evidence of active disease, and of this group, 10% had chronic, severe disease when surveyed every few months [2]. This category of disease severity is important because it may be associated with the later development of conjunctival scarring [1, 2]. In addition, individuals in this group may represent those who are at risk for the development of subsequent blindness in later adulthood [3].

Factors that were associated with severe disease include environmental and hygiene conditions [2]. In one longitudinal study, it was noted that persons with previous moderate or severe disease were more likely to continue with disease at subsequent points than were those with mild disease [4]. Continued disease severity was not solely attributable to sharing a bed with an active case.

There is also some evidence that differences in clinical response to chlamydial infection could be due to genetic variability. Differences in morbidity and mortality of *Chlamydia*-infected mouse strains were related to certain major histocompatibility complex (H-2) haplotypes and levels of heat shock protein (hsp) antibodies and differences in interferon-γ and tumor necrosis factor-α levels [5–10]. In humans, particular HLA haplotypes have been found to be independently associated with pelvic inflammatory disease and chlamydial infection and with conjunctival scarring in trachoma [11, 12]. In a recent article, the tumor necrosis factor-α allele, TNFA-308A, was significantly associated with trachoma with a higher risk for homozygosity and was not linked to HLA-A*6802 in scarring trachoma [13]. Since most persons who live in trachoma-endemic areas experience chlamydial ocular infection but relatively few develop long-term sequelae, it is possible that specific immunologic differences may define these subgroups.

We present a longitudinal study, using chlamydial DNA detection and sequencing methods, that identifies the association of persistent infection with severe disease and high chlamydial loads and the occurrence of different patterns of duration of infection between siblings living in a trachoma-endemic village.

**Materials and Methods**

**Study population.** *C. trachomatis* ocular infection and disease signs were assessed once weekly for 3 months for 33 families who had children <7 years old, living in a trachoma-endemic village. Fifty-three children ages 1–12 years were included: 32 girls and 21 boys. Thirteen families had >1 child, and 10 of 13 families had children with at least one occasion of infection.

A trained ophthalmic nurse examined both subtarsal conjunctivae from each subject. Clinical disease was scored using a simpli-
fied World Health Organization trachoma grading scheme: Intense inflammatory trachoma (TI with or without follicular trachoma [TF]) was present if ≥50% of deep tarsal vessels were occluded; TF was present if ≥5 follicles of ≥0.5 mm were seen. No signs of disease were graded [14]. The tarsal conjunctiva of the right eye was sampled with a cotton-tipped Dacron swab and placed in ligase chain reaction (LCR) buffer transport buffer (Abbott Laboratories, Abbott Park, IL). All subjects were treated with azithromycin (Zithromax; Pfizer Pharmaceuticals, New York, NY) at the end of the sampling period.

LCR. An LCR method (Abbott) for detection of the C. trachomatis cryptic plasmid DNA was used. In this test, sample values of ≥1 are scored as positive, according to the manufacturer’s instructions. LCR-positive samples were then dialyzed with molecular grade water by centrifugal filtration using polysulfone 30,000 molecular size cutoff filters (Ultrafree-MC; Millipore, Bedford, MA). This step was necessary to remove substances from the LCR transport buffer that would be inhibitory to subsequent polymerase chain reactions (PCRs).

HLA-DRA PCR. To assess the quality of DNA in the samples, a PCR for a 273-bp conserved area of the HLA-DRA gene was performed with primers [bp:348: 5′-3′:CCAGAGTTAAGCTTTGCTCACCGACA] and [bp:620: 5′-3′:CTTGGATAGGCTCTTCTCAAGC] (Genbank accession no. M60333) from the dialyzed LCR sample. Specimens from infection-negative and -positive periods were tested from children in the duration of infection subgroups. The effect of sample adequacy on Chlamydia LCR results and the effect of being in either a sporadic or persistent infection category on sample adequacy was determined.

PCR for sequencing. Primers that encompassed the whole hypervariable area of omp-1 were used to amplify a 1025-bp fragment from the dialyzed LCR sample. A hot-start method that uses wax beads (Ampliwax; Perkin-Elmer, Branchburg, NJ) was performed using a thermostate of 80°C for 5 min, followed by 94°C, 50°C, and 72°C for 1, 1, and 2 min, respectively for 30 cycles with a terminal extension at 72°C for 10 min. Five microliters of dialyzed sample was amplified with 2.5 U of Taq polymerase (Boehringer-Mannheim, Indianapolis, IN) per reaction in addition to the other PCR reagents, as previously published [15, 16]. Next, primers (bp:653: 5′-3′:GGGTATGGGTTAAGCTTTG and bp:1589: 5′-3′:GTGAGTGAAAGAAATAC) were used to generate a 937-bp fragment that was nested to the 1025-bp fragment in order to generate enough DNA for sequencing. Variable omp-1 domains (VD) 1 and 2 were sequenced in order to confirm the presence of persistent infection and to examine the relationship of infection patterns in siblings. The 937 bp DNA band was electroeluted from a 0.8% agarose gel slice for cycle sequencing by the fluorescent dyeoxy terminator method using an automated sequencer (Applied Biosystems, Foster City, CA) [17, 18]. VD1 and 2 nucleotide sequences were compared with published sequences of the reference strains of C. trachomatis [19]. An omp-1 genovar type number was assigned if there was a nucleotide substitution when compared with the reference strains. Nucleotide sequence information has been deposited with the International Sequences Databases (GenBank accession numbers: strain 1, variable domain 1 = AF015545; strain A1, variable domain 2 = AF015546; B-1 and B-2, variable domain 1 = AF015547; B-2, variable domain 2 = AF015548).

Adenovirus PCR. An adenovirus PCR for a 300-bp conserved area of the hexon gene was performed to rule out coinfection in some of the subjects [20]. Thirty samples from 13 children who had TI with or without TF without evidence of chlamydial DNA were tested in the adenovirus PCR. In addition, a random selection of 52 samples from 11 other children with follicular inflammation and chlamydial DNA at any time point were also tested. If a sample from a given time point was positive for adenovirus, then additional samples 2 weeks before and after the positive sample from the same child were also amplified for adenovirus.

Electrophoresis. Electrophoresis was performed through 7.5% polyacrylamide gels that had been stained with Sybr Green I (Molecular Probes, Eugene, OR). DNA bands from nested omp-1 HLA and adenovirus PCRs were visualized using a fluoroimager (Molecular Dynamics, Sunnyvale, CA).

Definitions of duration of infection and persistent disease. By definition, a child was categorized as having persistent chlamydial infection if there were ≥6 weeks of consistent LCR positivity without interruption with the same chlamydial genotype. Sporadically infected children were defined as those with LCR positivity occurring <6 weeks in addition to having intervening weeks of no LCR positivity. Two children who had infection that could only be observed for <6 weeks because the study period ended were not categorized.

A child was characterized as having persistent severe or persistent follicular disease if there were ≥6 weeks with TI with or without TF or ≥6 weeks with TF only, respectively. In addition, a child was defined as having no signs or signs of active disease sporadically if there were no signs of active disease for more than six visits.

Data analysis. χ² and Fisher’s exact tests were used to compare proportions. Means and 95% confidence intervals (CIs) are presented for LCR values. To account for the correlation among measures of the same individual over time, SEs for the reported mean LCR values were adjusted using the generalized estimated equation approach [21].

Results

Infection and disease subgroups. Sixty-six percent (35/53) of the children had at least one episode of infection, and the weekly patterns of duration of infection for 3 months were divided into subgroups as shown in figure 1. Subgroup I, the persistently infected group, consisted of 21 of 35 children with infection. Subgroup III and IV, the sporadically infected group, had 12 children. Compared with sporadically infected children, persistently infected children were more likely to have had persistent, severe disease (inflammation for ≥6 weeks; table 1). In addition, children with sporadic infections were more likely to have no signs of disease, whereas none of the persistently infected children were ever without signs of trachoma on examination. There was a significant association of being persistently infected and having persistent, severe disease concurrently (table 1; Fisher’s exact test, P = .04).

Children with active disease had significantly higher concomitant chlamydial loads than children with no signs (figure 2). The mean LCR values for positive samples were 2.7 (95% CI, 1.9–3.6) for children with no signs of active disease, 3.6,
quences were designated A1, B1, and B2 and are shown in figure 4 with their corresponding amino acid substitutions. Genotypes B1 and B2 contained a substitution in known B cell neutralizing epitopes in VD1, as indicated. Genotype remained constant in an infected child throughout an infection period. For children with persisting infection, 42.9% (9/21) had genotype A1, 42.9% (9/21) had B1, and 14.3% (3/21) had B2. Genotypes of 6 sporadically infected children included 3 with A1 and 3 with B2. For the remainder of sporadically infected children, it was not possible to generate sufficient amounts of DNA for sequencing. As indicated previously, only one genotype of A1, B1, or B2 was found in a given family. These results suggest that infection for an extended period of time might be due to failure to clear chlamydiae and not to reinfection. Although the sample size for the sporadically infected children is small, the data suggest that there was no association of a particular genotype with persistent infection as compared with sporadic infection.


cancer-PCR. A subgroup of samples was amplified for a conserved area of the adenovirus hexon gene, since adenovirus can cause self-limited follicular conjunctivitis and its prevalence was unknown in the study population. For the 82 samples of 26 children with follicular inflammation, amplified adenovirus DNA was detected in only 2 (2.4%) of the samples, each on a different child. Only 1 child was Chlamydia-positive. (95% CI, 1.3–3.8) for children with follicular disease only, and 4.0 (95% CI, 3.8–4.2) for children with severe disease. For the children with TF, the chlamydial load was distributed bimodally, as seen by LCR values that were >2 or negative (<1). Among all LCR-positive specimens (LCR value, >1), chlamydial loads were significantly higher in samples from children with persistent infection (LCR value, 3.8; CI, 3.7–3.9) than in samples from children in the sporadic infection groups (LCR value, 2.15; CI, 1.35–2.96) (figure 3). The presence of high chlamydial loads in children with persistent infection suggests that high levels of replicating organisms in this subgroup of children for long periods sustains severe disease.

Familial patterns. Thirteen families had >1 sibling, and 10 families (26 children) had at least one chlamydial infection (table 2). Of interest were families (1–6) who had at least 1 child with persistent infection. In 3 families, only 1 sibling was persistently infected. Sporadic infection patterns in the absence of persistent infection occurred in 4 families. In 3 of 4 families, only 1 sibling was sporadically infected with the other siblings uninfected. In addition, age in months, sex, and number of children in a family were not significantly associated with either duration of infection or disease severity (tables 2, 3). Chlamydial genotype remained constant within a family (see below). Although the sample size is small, the results suggest that familial exposure may not necessarily influence the same duration of infection in siblings.

Omp-1 DNA sequencing. Omp-1 VD1 and 2 regions of Chlamydia were sequenced in order to confirm that children who were LCR-positive for extended periods had the same genotype and were more likely to be persistently infected. Sequences were designated A1, B1, and B2 and are shown in figure 4 with their corresponding amino acid substitutions. Genotypes B1 and B2 contained a substitution in known B cell neutralizing epitopes in VD1, as indicated. Genotype remained constant in an infected child throughout an infection period. For children with persisting infection, 42.9% (9/21) had genotype A1, 42.9% (9/21) had B1, and 14.3% (3/21) had B2. Genotypes of 6 sporadically infected children included 3 with A1 and 3 with B2. For the remainder of sporadically infected children, it was not possible to generate sufficient amounts of DNA for sequencing. As indicated previously, only one genotype of A1, B1, or B2 was found in a given family. These results suggest that infection for an extended period of time might be due to failure to clear chlamydiae and not to reinfection. Although the sample size for the sporadically infected children is small, the data suggest that there was no association of a particular genotype with persistent infection as compared with sporadic infection.

Adenovirus PCR. A subgroup of samples was amplified for a conserved area of the adenovirus hexon gene, since adenovirus can cause self-limited follicular conjunctivitis and its prevalence was unknown in the study population. For the 82 samples of 26 children with follicular inflammation, amplified adenovirus DNA was detected in only 2 (2.4%) of the samples, each on a different child. Only 1 child was Chlamydia-positive. (95% CI, 1.3–3.8) for children with follicular disease only, and 4.0 (95% CI, 3.8–4.2) for children with severe disease. Samples taken 2 weeks before and 2 weeks after the adenovirus-positive samples were negative for adenovirus by PCR. For the children with TF, the chlamydial load was distributed bimodally, as seen by LCR values that were >2 or negative (<1). Among all LCR-positive specimens (LCR value, >1), chlamydial loads were significantly higher in samples from children with persistent infection (LCR value, 3.8; CI, 3.7–3.9) than in samples from children in the sporadic infection groups (LCR value, 2.15; CI, 1.35–2.96) (figure 3). The presence of high chlamydial loads in children with persistent infection suggests that high levels of replicating organisms in this subgroup of children for long periods sustains severe disease.

Relationship of sample adequacy to Chlamydia result and duration of infection group. In order to validate the assignment of children to duration of infection subgroups (figure 1), it was necessary to establish the adequacy of samples in terms of the presence of amplifiable human DNA. A subgroup of 160 samples from 22 children who had least 1 positive Chlamydia LCR was chosen for testing by conserved HLA-DRA PCR. Overall, 70% (112/160) of the samples tested were positive for the conserved HLA-DRA gene fragment. Of samples that were chlamydia-negative, 68.8% (64/93) were HLA-posi-

Table 1. Comparison of C. trachomatis infection duration with disease.

<table>
<thead>
<tr>
<th>Infection type</th>
<th>No. of children</th>
<th>No signs</th>
<th>Follicular</th>
<th>Severe</th>
</tr>
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<tbody>
<tr>
<td>No infection</td>
<td>18</td>
<td>22.2*</td>
<td>72.2</td>
<td>5.6</td>
</tr>
<tr>
<td>Sporadic infection</td>
<td>12</td>
<td>33.3*</td>
<td>58.3</td>
<td>8.3</td>
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<tr>
<td>Persistent infection</td>
<td>21</td>
<td>0</td>
<td>81.0</td>
<td>19.0</td>
</tr>
</tbody>
</table>

*Of 4 children, 1 never had any clinical trachoma signs.
† Of 4 children, 3 never had clinical trachoma signs.
Figure 2. Each point represents sample result. Clinical grade on x axis is as follows: inflammatory trachoma with or without follicular trachoma (TI ± TF) for intense inflammation, TF for follicles alone, and NS for no signs. LCR, ligase chain reaction.

Figure 3. Each point represents sample result. Data represent 250 samples of 21 children for persistent infection and 144 samples of 12 children for sporadic infection. LCR, ligase chain reaction.

tive compared with 71.6% (948/670) of Chlamydia-positive samples. In addition, 69.2% (45/65) of samples from 13 children who had persisting infection had adequate samples compared with 66.6% (56/84) of samples from 9 children who were

Table 2. Characteristics of sibling C. trachomatis infection duration.

<table>
<thead>
<tr>
<th>Family no.</th>
<th>Persistent</th>
<th>Sporadic</th>
<th>Uninfected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>6</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 3. Comparison of mean age and gender of children with C. trachomatis infection duration.

<table>
<thead>
<tr>
<th>Category</th>
<th>Mean age (years)</th>
<th>Male</th>
<th>Female</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent</td>
<td>5.1</td>
<td>21 (40)</td>
<td>31 (60)</td>
<td>1</td>
</tr>
<tr>
<td>Sporadic</td>
<td>5.4</td>
<td>3 (25)</td>
<td>9 (75)</td>
<td>0</td>
</tr>
<tr>
<td>Uninfected</td>
<td>5.3</td>
<td>9 (50)</td>
<td>9 (50)</td>
<td>0</td>
</tr>
<tr>
<td>Unclassified</td>
<td>4.5</td>
<td>2 (100)</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>5.1</td>
<td>21 (40)</td>
<td>31 (60)</td>
<td>1</td>
</tr>
</tbody>
</table>
placed in a sporadic infection group. In addition, the occurrence of an inadequate sample was a random event, in that there was no correlation between the occurrence of HLA negativity with a particular sampling week (data not shown). These results indicate that the inability to detect chlamydiae from a sample or the placement of a child in a sporadic duration of infection group was not necessarily due to an inadequate sample.

**Discussion**

These data suggest that persistent chlamydial infection is a common pattern for children living in a trachoma-endemic village. The association of persistent infection, and not sporadic infection, with high chlamydial loads indicates that chlamydial replication likely proceeds unchecked by the host. These results suggest that the significant association of severe disease with high chlamydial burden for prolonged periods directly contributes to disease severity. In addition, persistent chlamydial infection is not necessarily shared by family members.

It had been shown previously that treatment of *Chlamydia*-infected tissue-cultured cells with interferon-γ resulted in viable but aberrant and nonreplicating chlamydial forms that expressed hsp60 but not major outer membrane protein [25, 26]. It was hypothesized that this state of “persistent” infection as induced by interferon-γ could contribute to disease sequelae in animals due to the proposed involvement of hsp60 in ocular histopathology and delayed type hypersensitivity [27]. However, this type of persistent infection has not been demonstrated in animal models of chlamydial infection. The results from the present study indicate that infection can persist for at least three months. However, it would be important to demonstrate that this phenomenon occurs over a period of years in humans in order to validate the biologic concept of long-term persistence as related to immunopathology.

In a previous study, mediators of inflammation and scarring, such as interleukin-1β, tumor necrosis factor-α, transforming growth factor-β1, and interferon-γ activation of macrophages, which could also stimulate mediators of delayed type hypersensitivity, were shown to be significantly elevated in association with high chlamydial loads and severe follicular inflammation and scarring in trachoma [28]. Therefore, it is likely that these mediators, which are sustained in people who cannot control high levels of replicating organisms, contribute to the severe disease sequelae of scarring, trichiasis, and blindness. In a previous study, children whose clinical disease resolved spontaneously had greater lymphocytic proliferative response to chlamydial and recall antigens than those with persisting signs of trachoma [29]. Of interest, interferon-γ levels were similar in the 2 groups. It is not known whether a particular component of the cell-mediated response could be inhibitory to rapid clearance of organisms in children who have persistent infection.

Of interest in the present study were children who experienced infection intermittently or for short periods of time.
These children had much lower chlamydial loads and less severe disease. Sample DNA adequacy was similar in both the persistently and sporadically infected children, as indicated by the conserved HLA-DRA PCR, so bias of sample ascertainment was not an issue. In terms of sample adequacy, the PCR primers were chosen in an area of the HLA-DRA gene that is conserved in Caucasians. However, it is possible that for Tanzanians this is not the case, and so it would be helpful to repeat the PCR for samples from the remaining 30% who were negative by the HLA-DRA PCR using another conserved gene marker. In addition, we have demonstrated in unpublished studies that <5% of ocular samples have PCR inhibitors, indicating that sample inhibition would not have a substantial impact on detection of chlamydial from conjunctival samples. A biologic explanation of the observed result is that children with intermittent or sporadic infection could have experienced a much lower initial chlamydial infectious dose than children with persisting infection. However, it is also possible that quantitatively less of the infectious dose entered the host cell due to differential cell surface properties or that these children were more efficient in clearing chlamydial infection.

Risk factors for constant, severe trachoma in preschool children include family ownership of cattle and families with 1 or more siblings having trachoma [3]. However, the finding of variable duration of infection within families in the present study suggests that clearance of infection could be a variant characteristic that is not controlled solely by environmental exposure. Polymorphism at the HLA loci is one example of the effect of immunogenetic variation on disease manifestation. In a case-control study on unrelated persons, the HLA-A*6802 allele was significantly overrepresented in adults with trachomatous scarring resulting from chronic infection, but there was no significant association of class II alleles [12]. The frequency of particular HLA haplotypes or the linkage of non-HLA genes with HLA loci in Tanzanian families in association with duration of infection or disease in trachoma has not been documented. In our study, siblings with the same infection pattern should show more sharing of parental haplotypes than would be predicted by chance. In addition, if a disease-causing gene is not itself an HLA gene, but is close to the HLA gene, then a family study would be useful in identifying the association. Recently, microsatellite analysis of the human genome was used to identify an immunologic abnormality in children with persisting mycobacterial infection [30]. All affected children were homozygous (compared with unaffected parents and siblings) for a point mutation in the interferon-γ receptor 1, resulting in lack of expression of this receptor on the cell surface.

Another explanation for differences in duration of infection and disease severity is that certain C. trachomatis types could be more virulent. We did not find that a particular genotype was more predominant in children with persisting infection, even though two genotypes had a mutation in a B cell neutralizing epitope of the immunodominant omp-1 gene. However, this hypothesis has received some support from studies of chlamydial genital tract infection. In one study, F/G serovars were associated with significantly less urethral discharge in males [31]. However, omp-1 F genotype variants were significantly associated with pelvic inflammatory disease, whereas most infections with invariant F types or E genotypes were asymptomatic [32]. In addition, VD1/2 omp-1 polymorphism manifested by recombination and point mutation in C. trachomatis isolates from Nairobi prostitutes was seen as contributing to the endemcity of C. trachomatis [33]. However, a study on persons from 2 trachoma-endemic villages in the Gambia did not find major antigenic shift in omp-1 chlamydial genotypes over a 22-month period or any association of particular genovariants with disease severity or reinfection [34].

We were also interested in whether children who had persistent disease but no detectable chlamydiae could have been infected with another organism. Adenovirus ocular infection, which can cause a nonspecific follicular conjunctivitis and is found worldwide regardless of socioeconomic class, would be one candidate. The prevalence of adenovirus was not known in our population, and it was postulated that this organism might be a cofactor for severe disease. Recently, PCR has been demonstrated as a sensitive test for diagnosis of adenovirus keratoconjunctivitis [35, 36]. However, we did not find evidence by PCR that adenovirus was a significant cause of either ocular disease in the absence of chlamydiae or could act as a cofactor for disease in association with chlamydiae. It is possible that children in the persistent disease group who did not have detectable chlamydial DNA may have been in the healing stages versus those children who had detectable chlamydial DNA. More important, children who were negative for chlamydial DNA could represent children who had recently cleared chlamydial DNA.

It would be important to describe in future immunogenetic studies of trachoma those epidemiologic factors that have been shown to impact on disease. Some of the most important factors include distance to water, facial cleanliness, cattle herding and proximity to cattle, exposure to infected children due to child care, and crowding [37]. In addition, immunogenetic studies that would be linked to familial occurrence of scarring, trichiasis, and blindness in parents and grandparents as related to duration of infection and disease severity in children could address the issue of identifying children who may be at risk for developing trichiasis and blindness as adults.

In summary, this study has demonstrated that severe disease in trachoma is accompanied by persistent infection in association with high levels of chlamydiae and that duration of infection and disease varies between siblings. There was no evidence that specific omp-1 genotypes were associated with duration of infection, that genotypes changed during an infection period, or that adenovirus could be a cofactor in severe disease in trachoma. Further characterization of immunologic differences between subsets of children with various durations of infection would be beneficial for determining a targeted immunointervention strategy in children.
Acknowledgments

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References