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**Molecular Epidemiologic Study on Ocular
Chlamydial Infection among Children Living
in Trachoma Hyper-endemic Villages**

by

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DISSERTATION

**submitted to the School of Hygiene and Public Health
of The Johns Hopkins University in conformity
with the requirements for the degree of
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Abstract

Long-term effects of risk factors and DNA sequencing of *Chlamydia trachomatis omp-1* gene on active trachoma, severe inflammatory trachoma and *C.*

trachomatis infection were investigated in 289 children aged 1-2 years old in 1989, in Kongwa, Tanzania. One hundred seventy-six (61%) children were available for follow-up in 1995. Active trachoma and severe inflammatory trachoma at baseline predict active trachoma and severe inflammatory trachoma 6 years later after adjusting for age and gender, odds ratios 3.1(1.58-6.11) and 3.96(1.93-8.16), respectively. Familial cattle ownership, living greater than 2 hours away from a water source, and facial cleanliness at both time points were associated with the presence of active trachoma at both time points, odds ratios 2.58(1.15-5.79), 3.07(1.23-7.64), and 0.52(0.26-1.03), respectively. Noticeably, children who died during the follow-up period tended to have severe inflammatory trachoma at baseline in villages where trachoma was relatively less prevalent (OR: 3.75, 95% C.I.:1.09-12.98). DNA sequencing revealed 26 *C. trachomatis omp-1* genotypes of serovar A and 18 of B/Ba. *Omp-1* genotypes evolved temporally, and only 20% of genotypes in 1995 were present in 1989. Strains which had mutations in B-cell epitopes in 1989 did not predominate in the 1995 survey. However, the B/Ba genotypes exhibited increased variable domain 2 amino acid substitutions. Multitypic infection at baseline predicted subsequent severe disease and infection compared to monotypic infection, and was associated with familial cattle ownership. However, homotypic genotype infection with the

same strain six years later and severe trachoma was also observed. The results suggest that risk factor reduction interrupts the continuous episodes of re-infection with *C. trachomatis*, and that severe inflammatory trachoma could be a marker for identifying young children who were not likely to survive past preschool years. Both evolution of *omp-1* genotypes, and sequential, homotypic infection are converging mechanisms of trachoma endemicity.

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Moreover, I should like to say how grateful I am for people in Kongwa, Tanzania. They provided warm hospitality for me to accustom to Kongwa as soon as possible beyond language barrier. I do really hope this dissertation would

make significant contribution in trachoma research in order to make them suffer less from trachoma and the blinding complications in the near future.

Finally, I would like to thank my parents, my brother Yu-Sheng, and my wife Hsiu-wei for their fully supports from Taiwan.

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Specific Aims

This study includes two main manuscripts entitled, “Predictors of trachoma and infection in a six-year follow up of children aged one and two”, and “DNA sequencing of *Chlamydia trachomatis* major outer membrane protein-1 gene indicates separate mechanisms of trachoma endemicity”. The specific aims for these two papers are as follows:

1. to identify epidemiologic factors as predictive indicators for active trachoma, severe inflammatory trachoma, and *C. trachomatis* infection by performing a six-year follow-up of a cohort of children living in hyper-endemic trachoma villages;
2. to determine if trachoma and infection at an earlier time point were good predictors for trachoma and infection at a subsequent time point;
3. to examine the association between trachoma severity and childhood mortality;
4. to determine the degree of *C. trachomatis omp-1* polymorphism and evolution using a six-year follow-up of children living in hyper-endemic trachoma villages;
5. to determine how multitypic and homotypic *omp-1* genotype infection related to disease severity and “persisting” infection in a six-year follow-up;
6. to determine if specific epidemiologic variables correlate with multitypic *omp-1* genotype infection.

Background

Trachoma, which is caused by repeated infection by *Chlamydia trachomatis*, is an ancient disease described in Eber's Papyrus. It is characterized by inflammation of the lymphoid follicles and diffuse infiltration with papillae in the conjunctiva. The sequelae resulting from chronic inflammation include conjunctival scars, trichiasis, corneal complications and blindness (West and Bobo, 1995). Trachoma is viewed as a disease of poverty and poor hygiene and was one criterion used to reject people to immigrate to the United States in the early 20th century (Aronson, 1997). Even though trachoma has been eradicated in most developed countries, it is still the leading infectious cause of blindness in the world, especially in arid areas of developing countries (Grayston et al., 1985). Currently, it is estimated that at least five million people suffer from trachoma and six million people are blind because of trachoma. It is predicted that eight million people will be blind within the next 25 years (Schachter and Dawson, 1990; Thylefors et al., 1995). Moreover, trachoma hits the vulnerable groups in the poorest communities — women and children (Munoz and West, 1997). The number of cases of blindness will increase remarkably in the future in trachoma-endemic areas due to the fact that trachoma takes several decades to lead to blindness and there is a growing population of over 50 years old as the result of an increase of life expectancy. The public health impact and economic implications in the under-privileged communities is enormous.

Studies of trachoma and *Chlamydia trachomatis* in the past decades have provided some understanding of the etiology and epidemiology of the disease. Epidemiologic studies have described the prevalence and distribution of the disease and have identified the risk factors of infection and disease. In addition, there have been trachoma vaccine clinical trials on humans, conducted in the 1950s and 60s, but have failed to demonstrate efficacy against re-infection (Woolridge et al., 1967). The ocular model in the chimpanzee and other animal models have shown preliminary immune response profiles for primary and secondary infection (Taylor, 1985). With such advances in biomedical technology, research in this field also has experienced major breakthroughs. Some genes have been sequenced in all serovar types such as major outer membrane protein (MOMP) gene (*omp-1*), which is thought to be one of the major vaccine candidates for trachoma (Stephens et al., 1987; Yuan et al., 1989). Moreover, several B or T cell epitopes have been identified on the variable domains of omp-1 gene, which can be used to develop new generation of trachoma vaccine (Morrison et al., 1992). In addition, cytokines and T cells (Th1, Th2 and CD8 cells) are now the focus of research of effective immune response against *Chlamydia trachomatis* infection (Cotter and Byrne, 1996). Hygiene behavior modification in communities such as face-washing has been shown to be a significant factor in reduction of trachoma endemicity (West et al., 1995). Molecular epidemiologic studies have also been implemented to correlate characteristics of the etiologic agent with disease severity and to evaluate

transmission pattern within households and communities (Bailey et al., 1994; Hayes et al., 1995; Bobo et al., 1997). In the field of treatment, azithromycin has been found to be very potent and a promising antibiotic that could replace the topical tetracycline treatment (Bailey et al., 1993a). However, eradicating or even controlling trachoma seems to be an unattainable goal in hyper-endemic areas because of the lack of resources, utilization and integration of health services.

This literature review will summarize the etiology, clinical manifestations and complications, pathogenesis and immunology of chlamydial infection, diagnosis of chlamydial infection, epidemiology, treatment, and finally, prevention and control of trachoma.

Chlamydia trachomatis

The genus *Chlamydia* are obligate, intracellular, Gram-negative bacteria. They have a unique biphasic growth cycle that relies on host-derived ATP to synthesize chlamydial proteins. There are four species of the genus *Chlamydia*. *C. psittaci* is a common pathogen of birds and animals, but can occasionally cause human disease, psittacosis. *C. pneumoniae* causes respiratory tract diseases and is also thought to be associated with atherosclerosis. *C. pecorum* is found in ruminants and is associated with infectious polyarthritis, encephalitis, pneumonia and diarrhea. Finally, there is *C. trachomatis* which is described in detail below (Peeling and Brunham, 1996).

There are two human biovar types, biovar trachoma and biovar LGV (lymphogranuloma venereum) and one mouse biovar type of *C. trachomatis*. In addition, at least 18 serovar types, which are defined by microimmunofluorescence (MIF) testing, have been identified so far (Wang and Grayston, 1991). Serovar A, B, Ba and C are associated with trachoma; serovar D, E, F, G, H, I, J, K are linked to the urogenital infections; and biovar LGV, including serovar L₁₋₃, causes more invasive sexual transmitted disease, LGV. However, through antigenic relatedness and sequence analysis on omp-1 gene, these serovars can be classified into three serogroups: the B-complex serogroup (serovars B, Ba, D, Da, E, L₂ and L_{2a}), the intermediate complex serogroup (serovars F and G), and the C-complex serogroup (serovars A, C, H, I, Ia, J, and L₃) (Yuan et al., 1989).

Chlamydial Development Cycle

Chlamydia has two phases in its life cycle, an elementary body (EB) which is small (300 to 400 nm), infectious but metabolically inactive and a reticulate body (RB) which is larger (800 to 1000 nm), metabolically active but noninfectious. The first step in the life cycle occurs when the EB attaches to surface squamocolumnar epithelial cells through specific ligand-receptor binding. However, should be noted, that chlamydial adhesins and cell receptors have yet to be identified with certainty. Then, the EB enters the host cell through two possible mechanisms, phagocytosis or endocytosis. After internalization, vesicles containing EBs do not fuse with endosomes. Instead, the EB differentiates into

RB, which in turn, divides by binary fission. After 18 to 20 hours of infection, RBs begins to differentiate into EBs. Before rupturing the cell and releasing mature infectious EBs capable of infecting susceptible cells, the endosome membrane may expand to form an inclusion occupying over three-quarters of the cell. The growth cycle is completed when the host cell lyses or the intact inclusion is expelled in order to release EBs to start another infectious process.

Major Outer Membrane Protein(omp-1) Gene

Chlamydia possesses one of the smallest prokaryotic genomes, approximately 660 megadaltons with a cryptic 4.4 megadalton plasmid. There are about 800 genes in chlamydia. The most well characterized gene is one that encodes for the major outer membrane protein (MOMP), *omp-1* gene. From its sequence, it has been deduced that there are four hyper-variable domains (VD), and subtype specificity is located in these regions (Yuan et al., 1989). Most variability occurs in VD I, II and IV. In addition, the neutralization antibody epitopes are also found in these regions (Morrison et al., 1992). In animal models, partial protection against reinfection has been demonstrated through immunization of MOMP extract to guinea pigs (Batteiger et al., 1993).

Omp-1 diversity

Allelic polymorphism of the single copy of the *omp-1* gene has been thought to account for the antigenic variation of *C. trachomatis* (Stephens et al., 1987; Brunham et al., 1994). It has been suggested that *omp-1* remains stable in serotyping properties without major antigenic shift (Morrison et al., 1992). A

number of studies have investigated *omp-1* variants of *C. trachomatis* in different populations and geographic settings (Dean et al., 1992; Hayes et al., 1992; Brunham et al., 1994; Dean et al., 1995; Frost et al., 1995; Lampe et al., 1995; Brunham et al., 1996; Bobo et al., 1997; Dean and Millman, 1997). A relatively wide antigenic diversity of *omp-1* has been found in trachoma and sexually transmitted diseases (STD) core populations, whereas little heterogeneity has been found in non-STD core population. Lampe et al. has found little *omp-1* VD sequence variation in clinical isolates from patients with upper or lower genital tract infection (Lampe et al., 1993). In contrast, one study in STD core groups in Nairobi, Kenya, has indicated that the tempo of genetic change for *C. trachomatis* is very dynamic, and the *omp-1* locus is highly polymorphic with 51% major antigenic shift and 49% minor antigenic drift (Brunham et al., 1994).

It has been thought that recombination between different *omp-1* genes leads to major antigenic shifts, but, it is not very likely to occur because *C. trachomatis* has a single copy of *omp-1* gene in its chromosome. However, Hayes et al. identified an isolate with a novel *omp-1* gene which might have resulted from the natural recombination event between serovar L₁ and L₂ (Hayes et al., 1994).

Clinical Presentation of Trachoma and Its Sequelae

During childhood, active trachoma is characterized by lymphoid follicles, inflammation and diffuse infiltration with papillae on the conjunctiva. Follicles,

which are yellow or white with greater than 0.5 mm round spots are avascular lymphoid tissues with infiltration of B cells and T cells. Papillae are small red dots in the superior tarsal conjunctiva and are more pronounced in the presence of secondary bacterial infection. Severe inflammatory trachoma, which is seen in 10% or more of children in trachoma-endemic areas, presents severe inflammation and thickening of the conjunctiva obscuring over 50% of the deep tarsal vessels. Necrosis of follicles may lead to scarring which can appear as small, stellate spots or can be severe as a “basket weave” in the conjunctiva. Severe scarring can contract the tarsal plate and rotate the eyelashes inward. By adulthood, this can lead to trichiasis which is the presence of at least one eyelash rubbing on the eyeball. Corneal opacity appears when eyelashes turn in and then abrade the cornea. Blindness due to trachoma occurs if corneal scarring and opacification is present (West and Bobo, 1995).

Thylefors et al. proposed a simplified system to assess trachoma and its complications (Thylefors et al., 1987). The simplified WHO grading system is as follows:

Normal Conjunctiva The upper tarsal conjunctiva is pink, smooth, thin, and transparent.

Follicular Trachoma (TF) TF is defined by five or more ≥ 0.5 mm follicles in the upper tarsal conjunctiva. Follicles are round lumps or spots that are paler than the surrounding conjunctiva.

Intense Inflammatory Trachoma (TI) TI is defined by pronounced inflammatory thickening of the upper tarsal conjunctiva that obscures more than half of the normal deep tarsal vessels. Diffuse inflammatory infiltration, oedema, or enlargement of vascular tufts with or without follicles appears in the conjunctiva.

Trachomatous Scarring (TS) TS is defined by the presence of scarring in the tarsal conjunctiva. Scars are seen as white lines, bands, or sheets (fibrosis) in the tarsal conjunctiva. Sometimes, diffuse fibrosis can also obscure the tarsal vessels. Therefore, one needs to be more careful to distinguish these scars from TI.

Trachomatous Trichiasis (TT) TT is defined by the presence of at least one eyelash rubbing on the eyeball.

Corneal Opacity(CO) CO is defined by visible corneal opacity over the pupil.

Each category represents relevance for the public health impact by trachoma on a population. If high prevalence of TI is found in a population, *C. trachomatis* is considered to be highly active in this population, and a prompt intervention should take place. The high prevalence of TT which represents *C. trachomatis* activity in the past indicates the need for surgical services. The prevalence of CO indicates the potential impact of trachomatous blindness for the population (Munoz and West, 1997).

Binocular loupes (X2.5) should be used on an eye examination for trachoma. Photographs of tarsal conjunctiva have been used for trachoma grading in field studies. West et al. have shown high reliability of photograph grading, with percent agreement > 85% and kappa statistic > 0.7, compared to clinical grading in the field (West and Taylor, 1990). The simplified WHO trachoma grading system has been applied in several studies, and it has been shown to be a rapid and reliable assay for assessing the magnitude of the problem of trachoma.

Laboratory Diagnosis

Detection of ocular C. trachomatis infections

Laboratory diagnosis for ocular chlamydial infection include both culture and non-culture methods. Non-culture methods consist of antigen and nucleic acid detection methods. Serologic tests for *C. trachomatis* infection in trachoma are generally not useful because antibodies against *C. trachomatis* are long-lived and a positive serologic test will not distinguish a previous from a current infection (Black, 1997).

(1) Culture Methods

Traditionally, laboratory diagnosis of *C. trachomatis* infections in trachoma relies on cell tissue culture on McCoy cells. Definitive diagnosis is made by staining the McCoy cells with direct fluorescent antibody to species-specific MOMP and looking for inclusions. Due to its extremely high specificity, (nearly 100%), it is valued as the gold standard for detection of *C. trachomatis*.

However, the culture only detects viable infectious chlamydial elementary bodies and it has relatively low sensitivity when compared with nucleic acid amplification methods. In addition, strict cold chains of specimen transportation and storage conditions are required. One study has shown that *C. trachomatis* was not detected by culture method even in some severe trachoma patients (Taylor et al., 1989a). Nevertheless, organisms can be preserved from culture for genotyping, antimicrobial susceptibility testing or morphologic studies.

(2) Non-culture Methods

The non-culture methods include non-nucleic acid amplification methods, such as direct fluorescent antibody (DFA) tests and EIA, and DNA amplification methods.

(a) Non-nucleic acid amplification methods

Direct fluorescent antibody (DFA) test is one of the most useful diagnostic techniques, with a sensitivity over 80% and specificity over 98%. In addition, it is less time consuming and does not require cold-chain during transport. The DFA test can detect *C. trachomatis* in direct conjunctival smears on a slide by using direct fluorescent antibody microscopy. Monoclonal antibodies to the species-specific epitope of MOMP are preferred for detecting *C. trachomatis* infections by the DFA test. There are some disadvantages to using DFA testing. It is laborious and requires highly trained and experienced personnel. In addition, an adequate specimen with columnar epithelial cells is required for an optimal result.

Enzyme-linked immunosorbant assay (EIA) is another diagnostic test for *C. trachomatis*. It is based on the detection of all species of chlamydia lipopolysaccharide (LPS) which has higher solubility and quantity than MOMP. Most EIAs are less sensitive than “culture-based methods” with a sensitivity from 60% to 75%, and specificity of approximately 97% (Black, 1997). One major disadvantage, however, is a higher false-positive rate due to cross-reaction with the LPS of other gram-negative bacteria. Therefore, this technique is not widely used to detect trachoma infections because other bacteria also infect the conjunctiva.

(b) Nucleic acid amplification

Nucleic acid amplification technology has also been used to detect *C. trachomatis* infections. Polymerase chain reaction (PCR) and ligase chain reaction (LCR) are very promising because nucleic acid amplification methods are extremely sensitive and specific. In trachoma investigations, both PCR and LCR have been evaluated for detection of *C. trachomatis* infections at both ocular and nasal sites. Data have shown that chlamydial DNA load is positively correlated with disease severity and persistent infection. In addition, high rates of inapparent infection with *C. trachomatis* in a trachoma endemic area were detected by PCR. Although a false-positive result might be due to contamination, these can be minimized if proper practices are followed in the laboratory. In addition, false-negative results might be due to the presence of substances in the specimens that are inhibitory to nucleic acid amplification. It has been shown that

<5% of ocular samples have PCR inhibitors, indicating that it does not have substantial impact on the detection of *C. trachomatis* from conjunctival specimens (Bobo et al., 1997).

***C. trachomatis* typing**

Serovar-specificity of *C. trachomatis* is due to the serovar-specific epitopes in MOMP. Serovar typing of *C. trachomatis* is determined by MIF test which is sensitive and the only serologic test for detection of species- and serovar-specificity. Recently, *omp-1* gene typing of *C. trachomatis* based on nucleotide sequencing has been used to understand the epidemiology and pathogenesis of chlamydial infection (Dean et al., 1992; Bailey et al., 1994; Hayes et al., 1995; Bobo et al., 1997).

Immunology

Epidemiologic studies have shown that *C. trachomatis* infection and active trachoma decrease with age in the trachoma endemic areas (West et al., 1991b; Bailey et al., 1996). Data from trachoma vaccine trials in the 1960s and animal challenging studies have demonstrated that protective immunity against *C. trachomatis* was induced (Wang and Grayston, 1967; Woolridge et al., 1967). Currently, MOMP is a primary target antigen for chlamydial vaccine development. Neutralizing antibodies against MOMP have shown to protect against infection (Caldwell and Perry, 1982; Zhang et al., 1987). However, protective immunity is short-lived and homologous-specific, as seen from

multiple re-infections with the same or different serovar. This phenomenon of repeated infections with *C. trachomatis* in trachoma endemic areas suggests the absence of long-term protective immunity. Interestingly, in trachoma endemic areas where everyone is exposed to *C. trachomatis*, a substantial portion of people are less likely to have repeated *C. trachomatis* infection and trachoma while a significant subset of people suffer from frequent re-infections which lead to severe sequelae. Evidence has shown that the genetic factors of the host may participate in persistent *C. trachomatis* infection (Bobo et al., 1997). Delayed-type hypersensitivity response induced by chlamydial heat shock protein (HSP60) in re-infection is thought to be involved with the development of histopathology (Morrison et al., 1989a; Morrison et al., 1989b). It is thought that severe inflammation and tissue damage in chlamydial infection is immune-mediated.

It has been suggested that local secretory IgA (sIgA), rather than IgG, is protective from infection (Bailey et al., 1993b). In addition, suppression of chlamydia-specific IgA antibody-secreting cells has been observed in people with intense trachoma (Ghaem-Maghami et al., 1997). It is also thought that cell-mediated immunity (CMI) plays a principle role as seen B-cell in deficient mice that are capable of clearing infection (Ramsey and Rank, 1991). In the murine model, interferon- γ produced by T-cells and natural killer (NK) cells participate in the elimination of infected cells and clearance of *C. trachomatis* (William et al., 1993). In studies of adoptive transfer experiments and monoclonal Ab-mediated *in vivo* depletion, CD4⁺ cells have been identified as the predominant protective

T cell subset. In further analysis, Th1-type profile response such as IL-2, TNF- α and IFN- γ rather than Th2 profile has been found (Su and Caldwell, 1995).

Th1 vs Th2 response

Recent studies have focused on the cytokine profiles with disease severity in order to understand Th1/Th2 responses in chlamydial infection and immunopathology. Cytokines characteristic of a Th1 response, are IL-2, IL-12 and IFN- γ , and have been detected in individuals with intense inflammatory trachoma whereas a Th2 response, involving IL-4, IL-5 and IL-10, has not been detected in individuals with clinical symptoms of trachoma (Bobo et al., 1996). One case-control study in people with severe trachomatous scarring showed that hsp60 can increase the number of IL-4 producing cells and induce the production of IL-4-specific mRNA (Holland et al., 1996). To date, the data suggest that there are two CD4+ T cell subsets involved in different stages of infection, re-infection and disease severity. The immunological mechanism remains unclear.

Cytotoxic T Lymphocytes (CTL) response

Animal studies have demonstrated that cytotoxic T lymphocytes play a role in chlamydial infection. CTL lyse chlamydia infected cells *in vitro* as well as protect against *in vivo* challenge (Beatty and Stephens, 1994; Starnbach et al., 1994). Holland et al. detected peptide-specific CTL response from peripheral blood mononuclear cells (PBMC) only in individuals free from conjunctival scarring and in children resolving from infection (Holland et al., 1997).

Serovar-specific B-cell epitopes

Serovar-specific B-cell epitopes on the major outer membrane protein in *C. trachomatis* have been studied (Su et al., 1990b; Su and Caldwell, 1993; Batteiger, 1996; Batteiger et al., 1996). Epitopes map to VD I, VD II and VD IV while antigenic determinants have not been mapped to VD III (Morrison et al., 1992). In addition, monoclonal antibodies specific to epitopes are capable of neutralizing chlamydia infectivity (Caldwell and Perry, 1982). Therefore, these B-cell epitopes are major target antigens for the development of subunit chlamydial vaccine. However, it has been believed that *C. trachomatis* strains with mutations at the B-cell epitopes could escape neutralization and result in re-infection.

T-cell Epitopes

T-cell epitopes on the MOMP have been investigated (Su et al., 1990a; Allen et al., 1991). Surprisingly, T-cell epitopes have been identified on the non-surface exposed VD III as well as the other three surface exposed VDs. It has been shown that T cell's help for the immunodominant exposed VDs. B-cell determinants are elicited by incorporating the MOMP T-cell epitopes. Higher titered antibodies specific to the B-cell sites of the chimeric peptide can be produced after immunizing mice with chimeric peptides containing both T- and B-cell epitopes (Su and Caldwell, 1992). It is also postulated that mutation at the T-cell epitopes could result in re-infection or promote persistent infection with *C. trachomatis* and thus facilitate disease progression (Dean and Millman, 1997).

Therefore, it is important to include T-cell epitopes in a synthetic or recombinant subunit vaccine.

Persistent infection

Evidence of persistent infection has been shown *in vitro* and in epidemiologic studies. *In vitro*, nutrient deficiency, antimicrobial agents and interferon gamma can change chlamydial metabolism and development so that aberrant forms are produced, and they become dormant inside of infected cells. However, dormant chlamydia can still release some antigens and induce a chronic inflammatory response (Beatty et al., 1994). A subset of people with moderate to severe trachoma have no detectable viable organisms but have active chlamydial infection (Schachter et al., 1988). In addition, one study has shown that people with scarring are positive immunocytologically but are culture negative (Mabey et al., 1987). In a 3- month weekly follow-up, children with severe trachoma were associated with persistent *C. trachomatis* infection with higher chlamydial load (Bobo et al., 1997). Therefore, persistent infection as well as repeated infections are involved in establishing chronic inflammation that leads to severe sequelae in trachoma.

Epidemiology

Even though trachoma has been eradicated in most developed countries, at present, blinding trachoma is a major public health problem in various parts of geographic areas extending from North Africa and the sub-Saharan region,

throughout the Middle East, to the dryer regions of Indian subcontinent and southeast Asia. In addition, pockets of trachoma blindness exist in Latin America, Australia and the Pacific Islands (Dawson et al., 1981). Within these regions, trachoma is more prevalent in the poor, underdeveloped, and arid areas where water supplies are not sufficient.

Generally speaking, there are two different types of trachoma endemicity in the communities based on the prevalence of active trachoma: trachoma endemic and trachoma hyper-endemic. In the hyper-endemic communities, the prevalence of active trachoma is as high as 60 to 90 percent in preschool children (Courtright et al., 1989; West et al., 1991b). Preschool children are at highest risk for active trachoma and the prevalence generally decreases with age, especially by teenage (West et al., 1991b). Trachomatous scarring can be seen in school children and the prevalence increases with age. Late sequelae, such as trichiasis, entropion, and corneal opacity which indicate a high magnitude of trachoma endemicity in the past 15 to 40 years are common in this type of community (Courtright et al., 1989). In the trachoma endemic areas, the group at highest risk for active trachoma shifts to school children, and the prevalence of trachoma sequelae could be higher than that of active disease. Trachoma intervention on such communities should focus more on the complication of blindness (Munoz and West, 1997).

Age

Preschool children are a high risk group for *C. trachomatis* infection and active trachoma in the trachoma endemic areas. Several studies in Kongwa, Tanzania have provided us with most of our knowledge of the natural history of trachoma in the trachoma endemic areas. West et al. have shown 60% of preschool children had active trachoma and 10% had severe inflammatory trachoma (West et al., 1991b). Forty-eight percent of children aged 1-7 years old in one village were *C. trachomatis* positive by PCR (Bobo et al., 1991). Prevalence of active trachoma reaches a peak in preschool-aged children then declines rapidly after 10 years of age. Trachomatous scarring begins to appear in some school children and the prevalence increases with age, with it being 60% in people over 25 years old. In addition, trichiasis is only found in teenagers and adults and the prevalence reaches almost 20% in people over 25 years old (Taylor et al., 1992).

Sex

One cross-sectional study showed that females have 2-4 times the risk for follicular trachoma, trachomatous scarring, trichiasis and corneal opacity than males after adjusting for age (West et al., 1991b). Another study showed that female caretakers are more likely to have active disease than women who are not caretakers (Congdon et al., 1993). It is believed that child-care activities increase exposure to *C. trachomatis* for female caretakers for young children, who are the main reservoir of infection. In addition, one longitudinal study over one-year

period suggested an almost twofold increased risk of constant, severe trachoma for female children than male children (West et al., 1996).

Geographic Clustering

Clusters of trachoma has been demonstrated at the neighborhood and household level (Katz et al., 1988; Bailey et al., 1989; West et al., 1991b). The prevalence of active trachoma varied by villages from 38% to 95%. The cluster phenomenon is even more obvious within balozi, a geographically related ten-household unit (West et al., 1991b).

Water Availability

It has been observed that distribution and prevalence of trachoma is associated with arid and dusty areas where water resources are scarce (Prost and Negrel, 1989). Longer distance from a water source is associated with active disease and infection (Tielsch et al., 1988; West et al., 1989). In addition, active trachoma is also associated with the amount of water used on washing children in Gambia (Bailey et al., 1991). Less access to water (number of tube wells in wards) has also been identified as a risk factor for trachoma in Nepal (Katz et al., 1996). Although the importance of water supplies has been demonstrated in the prevention from trachoma, obstacles in environment factors, water engineering in arid and dusty areas as well as economic issues in trachoma-endemic developing countries make it very difficult to improve water supplies.

Cattle Herding

Cattle herding is believed to be associated with trachoma. Studies in Tanzania have showed that familial cattle ownership increases the risk of having trachoma and chlamydial infection in children (West et al., 1991a; West et al., 1995; West et al., 1996). This involves a complex cycle in which cattle droppings that tend to attract flies, place children at higher risk for exposure. This increase in chance of infection is also due to the decrease in water available necessary for hygiene in such arid communities. The study by West has demonstrated that cattle ownership is associated with a child less likely to have sustained facial cleanliness (West et al., 1995). However, cattle herding, which also represents higher socioeconomic status for households in those community, could confound the effect of socioeconomic status on trachoma or chlamydial infection. It is also believed that cattle could be a mark for families with traditional lifestyles who tend to have the poorest living condition (Munoz and West, 1997).

Type of Roof

The type of house roof has also been investigated for the association with trachoma in Tanzania. It is thought that a household with a tin roof indicates a higher socioeconomic status for the individuals than those without a tin roof. In addition, traditional thatch roof could be associated with higher density of flies inside of the house. One study has shown that the traditional type of roof is associated with trachoma and chlamydial infection (West et al., 1991c). Another study has shown that a tin roof on the house is associated with sustained facial

cleanliness for children (West et al., 1995). In one study in the same area a tin roof is not associated with higher incidence of trachoma (West et al., 1996).

Individual Hygienic Behaviors

Studies have indicated that facial cleanliness and other hygiene practices are associated with trachoma and chlamydial infection. Ocular discharge, nasal discharge, flies, dust and food on face have been used as indicators of lack of cleanliness in a child (West et al., 1991a). Among them, the first three characteristics are better indicators of active trachoma or severe inflammatory trachoma. Facial cleanliness is protective against active trachoma and severe inflammatory trachoma (Taylor et al., 1989b; West et al., 1991a). In a face-washing clinical trial, children with sustained facial cleanliness are less likely to have any trachoma and severe inflammatory trachoma one year later (West et al., 1995). In a study in Mexico, children who wash their face 7 or more times per week have significantly less trachoma than those who wash less often (Taylor et al., 1985). Furthermore, facial cleanliness can reduce the risk of constant, severe trachoma among children (West et al., 1996). Other hygiene behaviors and practices have also been examined. Handkerchief and towel use can reduce the risk for trachoma (Taylor et al., 1989b; West et al., 1991c). However, there are discrepancies on noseblowing onto clothes as risk for trachoma (Taylor et al., 1985; Tielsch et al., 1988).

Host Genetic Factors

Host genetic susceptibility plays a significant role on the clinical presentation and sequelae of trachoma. A case-control study of trachomatous scarring showed that cases are 1.9-fold times to have HLA-A28 antigen than controls. In addition, in subtyping of the HLA-A28 specificity, the A*6802 allele is significantly over-represented among cases (Conway et al., 1996). Finally, Conway et al. also showed that trachomatous scarring is independently associated with tumor necrosis factor alpha (TNF- α) promoter, TNFA-308A and TNFA-238A (Conway et al., 1997).

Virulence of C. trachomatis Strains

It has been postulated that certain strains of *C. trachomatis* are more virulent in clinical presentation than others. Two studies of genital infections have provided some evidence. One study showed men infected with serovar F/G were less likely to have symptoms of urethral discharge than those infected with other serovars (Van de Laar et al., 1996). Dean et al. found omp-1 F genotype variants were associated with upper genital tract infection, pelvic inflammatory diseases and histopathology compared to omp-1 F prototype. In addition, omp-1 E genotypes were more likely involved with asymptomatic diseases (Dean et al., 1995). However, another study by Bobo *et al.* did not support this postulation. The study did not find any particular genovar was associated with persistent infection over 3 months which was related to severe inflammatory trachoma (Bobo et al., 1997).

Control & Prevention

Although there are no community anti-trachoma campaigns in developed countries, trachoma disappears with economic development and associated socio-cultural and behavioral changes (Thylefors, 1985). However, trachoma and trachomatous blindness are still one of the major public health issues in the poor and under-privileged communities of developing countries where cultural and behavioral practices increase the risk of infection. Therefore, communities-based programs which include treatment and behavioral modification are needed to eradicate trachoma.

Improvement in Water Availability

It will be impractical to improve water availability in developing countries by hydraulic engineering in the arid areas because of lack sufficient funds. However, improvements in household water utilization is more practical for trachoma control in such settings.

Hygiene Behavior Modification

A study has demonstrated that facial cleanliness in children is protective for any trachoma or severe inflammatory trachoma for up to a year with 65% and 42% reduction, respectively (West et al., 1995). In a community clinical trial, intervention by face-washing has shown face-washing villages had lower risk of having severe trachoma one year later (West et al., 1995).

Antibiotic Treatment

Topical tetracycline 1% eye ointment or 1% oily drops 1-2 times per day for 4-6 weeks is effective in clearing infection and clinical symptoms. Although relatively inexpensive, good compliance is an issue. In addition, even with community-wide participation in trachoma endemic areas, topical tetracycline treatment only has a short-term effect. Data have shown the incidence of new infection one month after treatment stoppage is 21% (West et al., 1993).

There is a promising antibiotic against *C. trachomatis* called azithromycin. It is an azalide macrolide which can maintain long-term high tissue levels with only a single oral dose. The effect of azithromycin has been shown in a randomized controlled trial in treatment of trachoma, indicating that the one-dose azithromycin is as effective as topical tetracycline treatment to resolve clinical trachoma (Bailey et al., 1993a). However, one of the major disadvantages is the high cost of the antibiotic that makes it unaffordable for these under-privileged communities.

Vaccine

Although antibiotics are highly effective in controlling trachoma, there is still a need for an efficacious trachoma vaccine. Several trachoma vaccine trials were conducted in 1960's (Nichols et al., 1966; Dhir et al., 1967; Guerra et al., 1967; Woolridge et al., 1967; Nichols et al., 1969; Sowa et al., 1969). However, these vaccines, which used relatively crude, purified EB suspensions as antigen, only conferred a short-lived protective effect. Moreover, some vaccines seemed to increase serious complication of trachoma through enhanced hypersensitivity

reaction (Woolridge et al., 1967). Although, an EB-derived vaccine can induce a partial immunity in experimental animals, re-infection causes increased disease manifestations. In addition, the induced immunity was homotypic and heterologous infection in vaccinated animals led to substantially more severe disease (Brunham, 1994). In the past decade, understanding of the molecular antigenic structure of the chlamydial EB has generated new hope in the development of an effective chlamydia subunit vaccine.

MOMP has been the major vaccine antigen candidate. Several studies have tried to evaluate immunogenicity of the MOMP subunit vaccines which are oligopeptides, cloned recombinant fragments, or live recombinant vectors such as *Salmonella*, poliovirus or phage (Hayes et al., 1991; Murrin et al., 1993). The preliminary data were promising and encouraging in vitro.

However, there are several obstacles for an effective trachoma vaccine. First, mucosal immunity is needed against *C. trachomatis* infections. Conferring protective immune effectors such as sIgA to conjunctiva after immunization is necessary. Second, polymorphism of *omp-1* gene has been demonstrated in STD core group and trachoma endemic areas. An approach to incorporate cross-serovar or cross-genovar B-cell and T-cell epitopes in candidate antigen is needed to elicit immune responses which can protect from multiple serovars and genotypes.

Other chlamydial proteins have been considered in vaccine development, including heat shock protein (HSP70), MIP and exoglycolipid antigen. Of them,

a new vaccine design approach by using anti-idiotypic antibody to the exoglycolipid has shown induction of protective immunity against challenge in mice (Whittum-Hudson et al., 1996).

Molecular Epidemiologic Study on Ocular Chlamydial Infection Among Children Living in Trachoma Hyper-endemic Villages

In the beginning of this section, I will introduce the ethical issues in human subjects of this study, and explain the selection method of study population and the rationale. Then, this study will be presented in two manuscripts, entitled “Predictors of trachoma and *Chlamydia trachomatis* infection in a six-year follow up of children aged one and two” and “DNA sequencing of *Chlamydia trachomatis* major outer membrane protein-1 gene indicates separate mechanisms of trachoma endemicity”. Finally, other data which are not shown in the two manuscripts will be presented at the end of this section.

Issues in Human Subjects

This study was in accordance with the protocol (Research Project Notification Number: 95-06-02-05) which was approved by Joint Committee on Clinical Investigation at the Johns Hopkins Hospital in accordance with the Helsinki Declaration (Appendix 1). Verbal informed consent was obtained from participants’ parents. Children with active trachoma identified in the field were treated with topical tetracycline after sample collection. No adverse consequences or unexpected side effects are encountered in the course of study.

Study Population

In a 1989 face-washing intervention trial, 250 eligible households in each village containing at least one child aged 1-7 years were randomly selected after a complete census. Within each household, one child was randomly selected to participate in the study. A total of 289 children aged 1-2 years old from five villages that were part of a face-washing intervention program in 1989-90 were identified from files of the Kongwa trachoma project. These children were followed up in 1995 for this study. The results of this cohort of children are in manuscript one and two. The rationale to choose only children aged 1-2 years in this study includes that :1) children aged 1-2 years were at the highest risk in this region; 2) the first episode of disease or infection occurs in childhood; and 3) a higher follow-up rate can be achieved.

Another cohort of children aged 1-2 years old living in the same balozi (neighborhood unit) in 1995 were also selected in order to study the *C. trachomatis omp-1* polymorphism. The results of this cohort are in manuscript two. The selection method for this cohort of children was different from the first cohort. Random selection was performed from a complete census in each corresponding balozi in one village where children aged 1-2 years were plenty. All children aged 1-2 years in each corresponding balozi were enrolled in other four villages where the numbers of children aged 1-2 years old in balozi were not enough for the study.

Finally, DFA samples from 74 children aged 1-2 years in 1986 were used in the pilot study for the evaluation of sample adequacy.

Manuscript One

Predictors of Trachoma and *Chlamydia trachomatis* Infection in a Six-year Follow up of Children aged One and Two

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ABSTRACT

Long-term effects of risk factors on active trachoma, severe inflammatory trachoma and *C. trachomatis* infection were investigated in 289 children aged 1-2 years old in 1989, in Kongwa, Tanzania. 176 (61%) children were available for follow-up in 1995. Active trachoma and severe inflammatory trachoma at baseline predict active trachoma and severe inflammatory trachoma 6 years later after adjusting for age and gender, odds ratios 3.10(1.58-6.11) and 3.96 (1.93-8.16), respectively. Familial cattle ownership, living greater than 2 hours away from a water source and facial cleanliness at both time points were associated with the presence of active trachoma at both time points, odds ratios 2.58(1.15-5.79), 3.07(1.23-7.64) and 0.52(0.26-1.03), respectively. Noticeably, children from villages where trachoma was less common died in the six-year period tended to have severe inflammatory trachoma at baseline (odds ratio: 3.75, 95% C.I.:1.09-12.98). The results suggest that risk factor reduction interrupts the continuous episodes of re-infection with *C. trachomatis*, and that severe inflammatory trachoma could be a marker for identifying young children who were not likely to survive past preschool years.

INTRODUCTION

Trachoma is still one of the leading causes of blindness worldwide, especially in many developing countries in Africa, Asia, Latin America and Oceania (1, 2). It is believed that repeated ocular *Chlamydia trachomatis* infections result in a broad spectrum of clinical signs of trachoma from follicular trachoma or intense inflammatory trachoma in children, to conjunctival scarring in young adults, and trichiasis/entropion in older adults (3). The latter sign can lead to blindness in later adulthood. However, a considerable portion of people with chlamydial infection do not have any signs clinically, and cross-sectionally, a significant proportion of people with clinical trachoma do not have any laboratory evidence of *C. trachomatis* infection (4, 5). Studies have identified a subgroup of children in trachoma hyper-endemic communities with chronic, severe disease, and some with persistent infection (6, 7). It is suspected that this group of children is at high risk of developing severe sequelae in their later life. It is likely that multifactorial interactions between the host and chlamydia determine the clinical presentation and duration of trachoma in the face of infection (7).

Epidemiologic factors including demographic, socioeconomic, environmental, biologic and genetic factors, have been associated with clinical trachoma, and ocular chlamydial infection in cross-sectional studies, and in longitudinal studies with intermittent one year follow-up (6-11). However, the relationship of these epidemiologic factors to disease after a longer follow-up

period remains to be elucidated. Moreover, the relationships of trachoma severity to subsequent mortality in children has not been explored.

High mortality in very young children in sub-Saharan countries is still a tremendously devastating health issue for the under-served populations. Infectious diseases such as malaria, measles and AIDS, malnutrition and poverty have been linked to mortality of young children (12). Factors associated with childhood mortality, such as socioeconomic status and poor water sources, are also related to the presence of trachoma (13). In addition, host HLA haplotypes, that might have an impact on one's survival from other infections, may contribute to differential immune and clinical response to chlamydial infection and disease presentation (14, 15). It is unknown if active trachoma or severe inflammatory trachoma is associated with childhood mortality, and if the mechanism for such an association is direct or indirect. It is possible that either severe clinical manifestations of trachoma are linked to young children's mortality through other infectious processes, or that severe inflammatory trachoma could be an indicator for poor socioeconomic status in association with a specific host genetic makeup that predisposes to mortality.

The purposes of this study are 3 fold: 1) to identify epidemiologic factors as predictive indicators for the presence of active trachoma, severe inflammatory trachoma, and *C. trachomatis* infection six years later in a cohort of children in a hyper-endemic trachoma region; 2) to predict trachoma and infection in a later time point by previous trachoma and infection status; and 3) to examine the

association between trachoma severity and childhood mortality.

MATERIALS AND METHODS

Subjects. Kongwa, Tanzania is a trachoma hyper-endemic area where 60 percent of preschool children suffer from this disease (10). In a 1989 face-washing intervention trial, 250 eligible households in each village containing at least one child aged 1-7 years were randomly selected after a complete census. Within the household, one child each was randomly selected to participate in the study (16). All of 289 children aged 1-2 years old from five villages who were part of a face-washing intervention program in 1989-90 were identified from files of the Kongwa trachoma project. These children were traced, and 176 children were found in 1995 for follow-up studies.

In 1989-90, data were collected on age, sex, residential village and neighborhood, familial socioeconomic status represented by presence of a tin roof on the house and cattle ownership (16). Education level of the child's mother was categorized as either receiving any school education or never receiving any education. The time from the household to the nearest water source during the dry season was also determined as greater or less than 2 hours. In addition, facial hygiene status was evaluated by trained local interviewers as the presence of ocular or nasal discharge (16). Presence of clean face was defined as none or only one facial sign present. Additional data collected in 1995 included ethnicity (being Mkaguru). The reasons for children not to be available for follow-up were collected as refusal, death or migration.

Two photographs of the right tarsal conjunctivum of each child were taken before sample collection at each time point. In 1989-90, the tarsal conjunctivum of the right eye of each child was sampled with a cotton-tipped Dacron swab and rolled on direct fluorescent antibody (DFA) slides. In 1995, the ocular sample was placed in PCR transport buffer (4). Verbal informed consent was obtained from participants' parents. Children with active trachoma identified in the field were treated with topical tetracycline after sample collection. All study procedures were approved by Joint Committee on Clinical Investigation of the Johns Hopkins Hospital, in accordance with the Declaration of Helsinki.

Clinical grading of trachoma. Trachoma was graded from photographs of the right tarsal conjunctiva, using the W.H.O. simplified grading scheme (17): follicular trachoma if at least 5 follicles of > 0.5 mm were observed; severe inflammatory trachoma with or without follicles if 50% or greater of deep tarsal vessels were occluded; scarring if any scarring was evident. Active trachoma was defined as the presence of severe inflammatory or follicular trachoma. The photographs were graded by trained readers who were masked regarding village and laboratory identification number.

Laboratory (Appendix 2) Each DFA slide was scraped with an individual razor blade, and the scrapings were suspended in 100 μ l of water and boiled for 10 minutes (18). A diagnostic PCR-EIA assay for a conserved *omp-1* 280 bp gene fragment was performed (19). Processing controls for DNA contamination consisted of known DFA sample slides which were both DFA and PCR negative

from a previous study, and were included every tenth sample slide. The samples collected in 1995, which were in transport buffer, were processed as described previously (4). The diagnostic PCR-EIA assay was performed on all samples, using previously published methods (4, 19). Taq DNA polymerase (2.5 units) (Boehringer-Mannheim, Indianapolis, IN) was used in all PCRs.

Data Analysis. Characteristics of availability for follow-up were compared by contingency table analyses. McNemar test was used to examine the trend of trachoma in the follow-up children in the six-year period. In order to investigate the predisposition to trachoma and *C. trachomatis* infection in certain children, logistic regression models were used to predict active trachoma as a function of previous active trachoma status, or predict severe inflammatory trachoma as a function of previous severe inflammatory trachoma after adjusting for age and gender.

Multivariate logistic regression was used to examine the association between dependent variable and explanatory variables in the following analyses. In order to understand the long-term effect of risk factors, risk factors at baseline were used to predict the status of trachoma and infection in 1995. In addition, the association between risk factors in 1989 and active trachoma or severe inflammatory trachoma at both time points was examined. The effects on active trachoma and severe inflammatory trachoma at both time points of maintaining familial cattle ownership, the presence of tin roof, clean face and greater than two

hours to a water source during the dry season at two time points (1989-90, 1995), were also assessed.

In order to examine the impact of the modification of risk factors on disease at the follow-up, change in status of risk factors was used as a predictor. Active trachoma or severe inflammatory trachoma in 1995 in children was predicted by possession of these risk factors at baseline by logistic regression analysis after adjusting for age and gender.

The association between childhood mortality, trachoma and *C. trachomatis* infection at baseline was examined after adjusting for age, gender, familial cattle ownership and mother's education. Effect modification was further assessed using stratified analysis and multivariate logistic regression analysis by residential villages. Residential villages were stratified into two groups based on the prevalence of active trachoma in 1989 and geographic location: trachoma "high prevalence villages" with prevalence of active trachoma of 77% and 77%, and trachoma "low prevalence villages" with prevalence of 45%, 65% and 59% (Fig 1).

RESULTS

Characteristics of follow-up children compared to those lost to follow-up Sixty-one percent (176/289) of 1-2 year old children who were identified from files of the Kongwa Trachoma Project in 1989-90 were available in 1995. Characteristics of children who were not located tended to be different from those who were located (Table 1). Those children who were lost to follow-up tended not to have severe inflammatory trachoma, *C. trachomatis* infection, and to live in families who owned cattle, as compared to children who were followed up. None of the other epidemiologic characteristics were significantly different between children who were followed up in 1995 and children who were not. For the subset of 28 (10 %) children who were lost to follow-up due to death, 61% were 1 year old in 1989 compared to 38% age one year old who were available for follow-up in 1995 (Table 1). Death rates were higher in younger children, so this finding was expected. Two (0.7%) children refused to participate in 1995.

Prevalence trend in trachoma and C. trachomatis infection in six-year follow-up

The prevalence rate of active trachoma, severe inflammatory trachoma and *C. trachomatis* infection among the same children in 1989-90 and 1995 is shown in Table 2. There was a significant drop in the prevalence of active trachoma from 70 percent to 55 percent, while there was a slight drop in the prevalence of severe inflammatory trachoma from 33 percent to 25 percent. For 1989 samples, DNA was amplified from archival DFA slides, whereas for 1995 samples, DNA

was amplified on fresh specimens stored in buffer. So, we did not compare infection rate from baseline to follow-up.

Risk factors for trachoma and infection in 1989 and 1995 Table 3 illustrates the status of trachoma and four epidemiologic factors in the same children in 1989-90 and 1995. Among the 176 children who were available for follow-up, 44 percent had active trachoma at both time point, and 19 percent were free of active trachoma at both time points. A significant proportion (14 %) had severe inflammatory trachoma at both time points. Over 80 percent of children did not change status for familial ownership of cattle or for tin roof, indicating stability for this risk factor. However, there was 36% improvement of facial cleanliness, and 25% had better access to water at the later time point (Table 3).

Prediction of disease six years later by the status at baseline The 1989 disease status were used to predict these outcomes in 1995. Children with active trachoma and severe inflammatory trachoma in 1989 were more likely to have the same outcome in 1995 after adjusting for age and gender (Table 4).

Risk factors for trachoma in 1995 The association of active trachoma, severe inflammatory trachoma, and *C. trachomatis* infection in 1995 with epidemiologic variables at baseline is depicted in Table 5. Children who were living greater than 2 hours away from a water source in 1989 were more likely to have severe inflammatory trachoma. In addition, familial cattle ownership at baseline was marginally associated with the presence of trachoma.

Risk factors for trachoma at both time points Further analyses of risk factors for the presence of active trachoma, and severe inflammatory trachoma at both time points for children are shown in Table 6. Familial cattle ownership in 1989 was significantly associated with the presence of active trachoma at both time points, while facial cleanliness in 1989 was marginally protective against disease at both time points. In addition, living greater than 2 hours away from a water source in the dry season, and familial cattle ownership in 1989 were predictors for severe inflammatory trachoma at both time points.

The stability of the risk factors may be an issue in having disease or infection at two time points. Therefore, we conducted analyses for examining the effect of familial cattle ownership, having a tin roof on the house, facial cleanliness and living greater than 2 hours away from water source in the dry season at both time points after adjusting for other variables on trachoma and infection (Table 7). Familial cattle ownership and living greater than 2 hours away from water source in the dry season at both time points was significantly associated with the presence of active trachoma at both time points, while lack of facial cleanliness in 1989 and 1995 marginally increased the risk for the presence of active trachoma in 1989 and 1995. In addition, familial cattle ownership at both time points were predictors for the presence of severe inflammatory trachoma at both time points.

Reduction of disease by the change of risk factors In order to determine if the modification of risk factors can reduce the risk of having trachoma, familial cattle

ownership, living in a house without tin roof, living greater than 2 hours from water, or an unclean face at baseline were investigated. Children with an unclean face at baseline but who had a clean face at follow-up had reduced risk of severe inflammatory trachoma (OR = 4.03, 95% C.I.=1.02-15.93, $p=0.047$) compared to having an unclean face at both time points. Adjustments for age, gender and maternal education were made.

Child mortality and severe inflammatory trachoma Because 10 percent of children aged 1-2 years old in 1989-90 were known to have died by 1995, the association between trachoma, *C. trachomatis* infection and death of children was investigated. After adjusting for age, gender, familial cattle ownership and maternal education, there was a trend for active trachoma, severe inflammatory trachoma and *C. trachomatis* infection to predict the death of children. Severe inflammatory trachoma was the strongest predictor of mortality (Table 8). In particular, severe inflammatory trachoma was a strong predictor of mortality of children aged 1-2 in “low prevalence villages”.

DISCUSSION

Children with active trachoma, severe inflammatory trachoma or *C. trachomatis* infection in their early life are more likely to have trachoma and infection six or seven years later in this trachoma-endemic area. In these communities, almost every child will acquire ocular *C. trachomatis* infection and have mild to severe clinical trachoma in their early life. The prevalence, and duration of trachoma in children appears to decrease with age (10, 20). Reasons for this decline could be that the immune system in older children may generate some degree of protective immunity to chlamydial re-infection. It is also possible that there is less exposure to *C. trachomatis* with increased age, due to the change in risk factors in older children. In this study, we expected to see the decline of active trachoma six years later in children aged 1 and 2 years at baseline. However, certain children tend to suffer from this disease constantly, and do not lose clinical signs. In one previous longitudinal study with three time points in Gambia, persons with previous moderate or severe trachoma were more likely to have active disease at previous or subsequent points over a 20-month period than those with mild trachoma (21). We have demonstrated previously that subsets of children in this region tended to have persistent, severe disease over one year, and in another study to be infected for up to three months (6, 7). In this study, the presence of active trachoma and severe inflammatory trachoma at baseline was predictive of trachoma and severe inflammatory trachoma six years later.

Therefore, subgroups of children in this area are still at high risk of having disease.

One possible explanation for “persisting” disease is that infection at the second time point is just another re-infection and the child is at high risk for constant re-infection. In this case, chlamydial infection can be reduced through modification of behavioral, hygienic and environmental factors, or with antibiotic treatment (6, 16, 22-24). This data suggested that the risk of having trachoma was reduced by changing status of facial hygiene in a six-year period. In addition, we also demonstrated that maintaining risk factors at both time points, including familial cattle ownership, long distance to water source and unclear face, was associated with “persisting” disease six years apart. It is likely that interventions of improved water availability and increased facial hygiene can reduce repeated episodes of active trachoma. We would also expect that the risk of trichiasis and blindness could also be reduced in these individuals.

However, an additive explanation of “persisting” disease is that it represents persistent *C. trachomatis* infection with the same strain, and not exogenous re-infection. It has been demonstrated that persistent ocular *C. trachomatis* infection is associated with high chlamydial loads versus sporadic infection in associated with low chlamydial burden (7). In addition, interferon- γ can induce persistent chlamydial infection in tissue-cultured cells (25). Genotyping on the major outer membrane protein gene (*omp-1*) in 22 children with “persisting” infection demonstrated that almost half of the children had the

same *omp-1* genotype throughout an infectious period (26). These studies support the view that host-associated factors may interact with socio-environmental risk factors to maintain infection in this cohort.

In the present study, children's facial cleanliness and water availability over six years period improved at the later time point. Face-washing campaigns have demonstrated that it is possible to reduce unclean faces and severe trachoma in this area five years ago, and that this could be a major factor for improving children's facial hygiene (16). In addition, with increase of age, children were more likely to take care of themselves. Nevertheless, an long-term community-based intervention is still needed because *C. trachomatis* can strike back promptly (24). Since repeated re-infections or persisting infection with *C. trachomatis* infection could lead to severe sequelae of trachoma, it is very likely that impacting the sequelae of trachoma will only work using long-term, community-based intervention on elimination of *C. trachomatis*.

The results of this study indicate that having facial cleanliness can reduce the risk of severe disease in endemic settings. In addition, children whose mother went to school were 1.6-fold more likely to have clean face in 1989 (data not shown). In a case-control study in Gambia, families with trachoma used less water for washing children than those without trachoma (27). However, previous studies have demonstrated that water availability may not be the determining factor for water use in the household (10, 28). Therefore, increasing literacy and incorporating face-washing measures into school education should have a

substantial impact on facial cleanliness of young children over a long period to reduce trachoma endemicity in this area. Therefore, more effective trachoma control could be achieved if appropriate water usage programs are linked with improvement of water availability.

In this study, 10 percent of children aged 1-2 years old in 1989 were not available for follow-up in 1995 due to death. The overall children who died in the six-year period could be higher, since the information was unknown in children who were lost to follow-up due to migration. However, the similar distribution in age, gender, socioeconomic status (living in a house with tin roof), and maternal education between children who migrated and children who were available for follow-up, indicates that mortality in children who migrated could be low. In addition, severe trachoma and chlamydial infection were much lower in this group of children. Also, we observed the same trend in the association between child mortality and severe inflammatory trachoma using the group of follow-up children or the group of migrated children as the reference group. Therefore, loss to follow-up due to migration is probably not likely to be a significant factor to bias the association.

The association of child mortality and severe trachoma has not been reported. In our study, severe inflammatory trachoma was associated with death of children in “low prevalence villages” after adjusting for age, gender, socioeconomic factors (familial cattle herding and mother education), but not in “high prevalence villages”. Since the mortality rate was similar in both groups of

villages, it is unlikely that severe inflammatory trachoma per se is causal in the death of young children. In addition, there was no difference in loss to follow-up children by “prevalence villages”. One possible explanation is that children with severe inflammatory trachoma might tend to be more susceptible to other childhood diseases and have more serious complications. In the “low prevalence villages”, households of these children may more likely to be poorer or to have traditional practices which contributed to children’s death. On the other hand, the socio-economic status and practices in “high prevalence villages”, where over 90% were ethnic group Mkaguru, were more homogenous than those in “low prevalence villages”. In addition, near 80% of children had active trachoma at baseline in “high prevalence villages” which could dilute the effect by trachoma on a child’s death. Therefore, effect modification by “prevalence villages” on the association between severe inflammatory trachoma and child mortality was observed. Nevertheless, severe inflammatory trachoma could be a good and practical indicator for children aged 1-2 years who may be at increased risk of early death in trachoma endemic countries.

In summary, this study has demonstrated that risk factors can have cross-sectional as well as longitudinal associations with the presence of active trachoma, severe inflammatory trachoma and *C. trachomatis* infection in a trachoma endemic area. Moreover, this is the first report of an association between severe inflammatory trachoma and early childhood mortality. It will be

necessary to document other ongoing infectious and the presence of immune risk factors in order to clarify the association with childhood mortality.

FIGURE LEGENDS

Figure 1. Subdistricts within Kongwa district, Dodoma Region of Tanzania.
Study villages are indicated by †; * denotes the study administrative site.

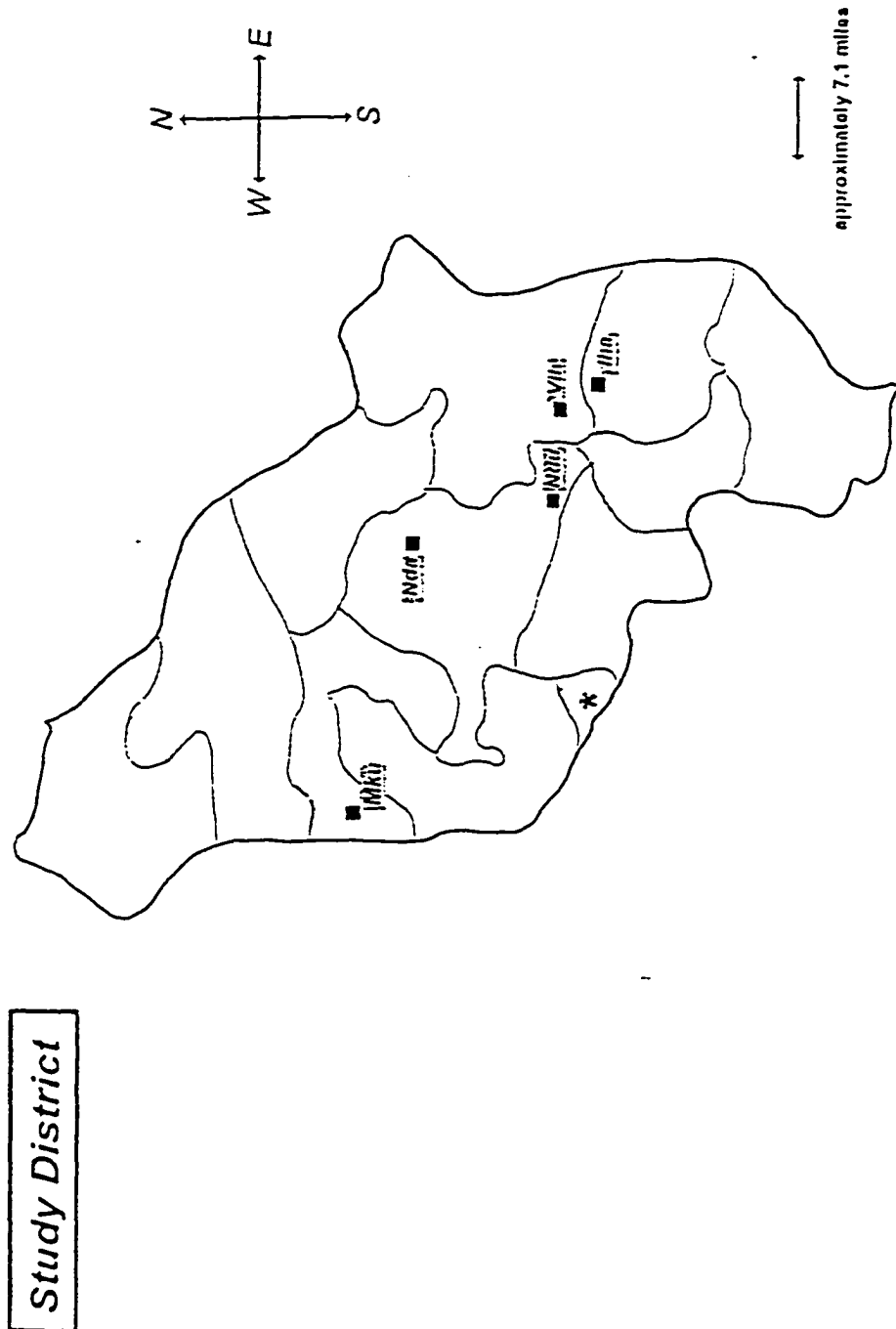


Table 1. Comparison of Characteristics by Availability for Follow-up

Variables in 1989		<i>Percent of children</i>		
		Available in 1995 (n=176)	Lost to follow-up ¹ (n=85)	Deceased (n=28)
Age (2 year versus 1 year)		62	55	39*
Gender	Female	55	45	54
	Male	45	55	46
Ethnic Group (Mkaguru)		69	nd ²	nd
Active Trachoma at baseline		70	58	68
TI ³		33	20*	39
<i>C. trachomatis</i> infection ⁴		27	14*	25
High Prevalence Villages ⁵		49	44	43
Familial Cattle Ownership		35	20*	18
Tin Roof		22	28	19
Clean Face		58	61	50
Water Source (>2 hr dry season)		43	41	39
Some Maternal Education		49	54	50

¹ Children who were not deceased² No data³ Severe inflammatory trachoma⁴ PCR detection on DFA slide samples⁵ High prevalence as defined by active trachoma

* Children who were available for follow-up as reference group, p<0.05

Table 2. Disease and Infection Prevalence in 1989-90 and 1995 in follow-up children

Characteristic	<i>Number (Percent)</i>	
	1989-90 (Age 1-2 years)	1995 (Age 6-8 years)
Active Trachoma	123 (70)	97 (55)*
TI ¹	57 (33)	44 (25)
<i>C. trachomatis</i> infection ²	-- ³	70 (40)

¹ Severe inflammatory trachoma

² PCR detection

³ Data were not comparable due to method of PCR detection, so it is not presented

* p<0.05 by McNemar test

Table 3. Comparison of Characteristics in 1989-90 with 1995 of 176 Children Available for Follow-up ¹

<i>Characteristic present in 1989-90 1995</i>		<i>Variables- Number (Percent)</i>					
		<i>Active Trachoma</i>	<i>TI ²</i>	<i>Cattle</i>	<i>Tin Roof</i>	<i>Clean Face</i>	<i>Water (>2hr)</i>
No	No	34 (19)	98 (56)	96 (55)	121 (69)	11 (6)	91 (52)
No	Yes	19 (11)	19 (11)	18 (10)	17 (10)	63 (36)	9 (5)
Yes	No	45 (26)	32 (18)	18 (10)	10 (6)	18 (10)	43 (25)
Yes	Yes	78 (44)	25 (14)	44 (25)	28 (16)	83 (47)	32 (18)

¹ Clean face and water > 2 hours was available for n=175, and TI for n=174.

² Severe inflammatory trachoma

Table 4: Prediction of disease status in 1995 among the follow-up children by the disease status in 1989

Characteristics(1989)			Characteristics(1995)		
		Number	Number (%)	OR (95% CI)	p-value
			Active Trachoma(1995)		
Active Trachoma	Yes	123	78(66%)	3.10(1.58-6.11)	0.001
	No	53	19(38%)	1.00	
			TI ¹		
TI ¹	Yes	57	25(44%)	3.96(1.93-8.16)	<0.001
	No	117	19(16%)	1.00	

Logistic regression analysis adjusting for age and gender

TI¹ = Severe inflammatory trachoma

Table 5. Predictors of the Presence of Disease or Infection in 1995 of 176 Follow-up Children

Characteristics(1989)	Category	Active Trachoma	Severe Inflammatory Trachoma ¹	<i>C. trachomatis</i> Infection ²
		Adjusted OR(95% CI)	Adjusted OR(95% CI)	Adjusted OR(95% CI)
Age	2 years	0.94 (0.49-1.79)	0.79 (0.38-1.63)	1.24 (0.65-2.38)
	1 year	1.00	1.00	1.00
Gender	Female	0.73 (0.39-1.38)	0.95 (0.46-1.97)	0.84 (0.45-1.59)
	Male	1.00	1.00	1.00
Ethnicity (Mkaguru)	Yes	1.20 (0.60-2.40)	1.88 (0.78-4.49)	1.60 (0.78-3.26)
	No	1.00	1.00	1.00
Cattle Ownership	Yes	1.82† (0.91-3.62)	1.17 (0.54-2.51)	1.66 (0.84-3.25)
	No	1.00	1.00	1.00
Tin Roof	Yes	1.39 (0.63-3.04)	1.45 (0.59-3.53)	1.25 (0.58-2.72)
	No	1.00	1.00	1.00
Clean Face	Yes	0.86 (0.45-1.64)	0.69 (0.33-1.45)	1.00 (0.52-1.92)
	No	1.00	1.00	1.00
Distance to Water	< 2 hours	1.73 (0.89-3.37)	2.15* (1.01-4.58)	1.40 (0.72-2.71)
	>= 2 hours	1.00	1.00	1.00
Maternal Education	Some	1.34 (0.71-2.52)	0.92 (0.45-1.91)	0.82 (0.43-1.55)
	None	1.00	1.00	1.00

Logistic Regression Analysis; *p<0.05 and †0.05<p<0.1

¹ 174 cases for severe inflammatory trachoma

Table 6. Predictors of the Presence of Disease at Both 1989-90 and 1995 of 176 Follow-up Children

Characteristics (1989)	Category	Active Trachoma	Severe Inflammatory Trachoma ¹
		Adjusted OR(95% CI)	Adjusted OR(95% CI)
Age	2 years	0.75 (0.38-1.47)	0.66 (0.26-1.66)
	1 year	1.00	1.00
Gender	Female	0.70 (0.36-1.36)	1.00 (0.39-2.54)
	Male	1.00	1.00
Ethnicity (Mkaguru)	Yes	1.70 (0.82-3.53)	2.57 (0.68-9.67)
	No	1.00	1.00
Cattle Ownership	Yes	2.66* (1.32-5.36)	2.28† (0.87-5.97)
	No	1.00	1.00
Tin Roof	Yes	1.25 (0.56-2.81)	1.07 (0.30-3.76)
	No	1.00	1.00
Clean Face	Yes	0.51† (0.21-1.01)	0.51 (0.20-1.34)
	No	1.00	1.00
Distance to Water	< 2 hours	1.70 (0.86-3.35)	3.00* (1.08-8.31)
	>= 2 hours	1.00	1.00
Maternal Education	Some	1.48 (0.76-2.87)	0.83 (0.32-2.13)
	None	1.00	1.00

Logistic Regression Analysis: * $p < 0.05$ and † $0.05 < p < 0.1$

¹ 174 cases for severe inflammatory trachoma

Table 7. Likelihood of the Presence of Disease for Both 1989-90 and 1995 in Association with Categorical Variables Present at Both Time Points of 176 Follow-up Children

Characteristics (1989)	Category	Active Trachoma	Severe Inflammatory Trachoma ¹
		Adjusted OR(95% CI)	Adjusted OR(95% CI)
Age	2 years	0.84 (0.42-1.66)	0.67 (0.27-1.65)
	1 year	1.00	1.00
Gender	Female	0.59 (0.30-1.14)	0.84 (0.34-2.07)
	Male	1.00	1.00
Ethnicity (Mkaguru)	Yes	1.43 (0.67-3.04)	2.48 (0.65-9.45)
	No	1.00	1.00
Cattle Ownership	Yes	2.58* (1.15-5.79)	2.38† (0.90-6.31)
	No	1.00	1.00
Tin Roof	Yes	0.90 (0.35-2.27)	0.27 (0.03-2.29)
	No	1.00	1.00
Clean Face	Yes	0.52† (0.26-1.03)	0.59 (0.23-1.50)
	No	1.00	1.00
Distance to Water	< 2 hours	3.07* (1.23-7.64)	1.06 (0.35-3.25)
	>= 2 hours	1.00	1.00
Maternal Education	Some	1.42 (0.74-2.72)	0.83 (0.34-2.05)
	None	1.00	1.00

Logistic Regression Analysis; *p<0.05 and †0.05<p<0.1

¹ 174 cases at both time points for active trachoma, and 172 cases for severe inflammatory trachoma

Table 8. Childhood Mortality Predicted by the Association of Disease and Infection in 1989-90 in High and Low Prevalence Villages

Characteristics	Total (n=289)			High Prevalence Villages (n=135)		Low Prevalence Villages (n=154)	
	Crude OR	Adjusted OR	95% CI	Adjusted OR	95% CI	Adjusted OR	95% CI
Active Trachoma	1.09	1.23	0.52-2.91	0.42	0.11-1.62	2.88†	0.85-9.78
TI ¹	1.61	1.77	0.76-4.12	1.16	0.33-4.11	3.75*	1.09-12.98
<i>C. trachomatis</i> Infection ²	1.12	1.27	0.50-3.22	1.30	0.35-4.80	1.55	0.37-6.45

Logistic Regression Analysis, adjusted for age, gender, cattle herding and maternal education; * $p < 0.05$ and † $0.05 < p < 0.1$

¹ Severe inflammatory trachoma

² PCR detection

References

1. Munoz B, West S. Trachoma: the forgotten cause of blindness. *Epidemiologic Reviews* 1997;19:205-217.
2. Thylefors B, Negrel A-D, Pararajasegaram R, Dadzie K. Global data on blindness. *Bull World Health Organ* 1995;73:115-121.
3. Grayston J, Wang S-P, Yeh L-J, Kuo C-C. Importance of reinfection in the pathogenesis of Trachoma. *Rev Infect Dis* 1985;7:717-725.
4. Bobo L, Munoz B, Viscidi R, Quinn T, Mkocho H, West S. Diagnosis of *Chlamydia trachomatis* eye infection in Tanzania by polymerase chain reaction/enzyme immunoassay. *Lancet* 1991;338:847-850.
5. Bailey R, Hayes L, Pickett M, Whittle H, Ward M, Mabey D. Molecular epidemiology of trachoma in a Gambian village. *Br J Ophthalmol* 1994;78:813-817.
6. West S, Munoz B, Lynch M, Kayongoya A, Mmbaga B, Taylor H. Risk factors for constant, severe trachoma among preschool children in Kongwa, Tanzania. *Am J Epidemiol* 1996;143:73-78.
7. Bobo L, Novak N, Munoz B, Hsieh Y, Quinn T, West S. Severe disease in children with trachoma is associated with persistent *Chlamydia trachomatis* infection. *J Infect Dis* 1997;176:1524-1530.
8. West S, Congdon N, Katala S, Mele L. Facial cleanliness and risk of trachoma in families. *Arch Ophthalmol* 1991;109:855-857.
9. West S, Rapoza P, Munoz B, Katala S, Taylor H. Epidemiology of ocular chlamydial infection in a trachoma-hyperendemic area. *J Infect Dis* 1991;163:752-756.
10. West S, Munoz B, Turner V, Mmbaga B, Taylor H. The epidemiology of trachoma in central Tanzania. *Int J Epidemiol* 1991;20:1088-1092.
11. Katz J, West K, Khattry S, et al. Prevalence and risk factors for trachoma in Sarlahi district, Nepal. *Br J Ophthalmol* 1996;80:1037-1041.
12. Brockerhoff M. Child mortality in East Africa: the impact of preventive health care. New York: The Population Council, 1995.
13. Marx R. Social factors and trachoma: a review of the literature. *Soc. Sci. Med.* 1989;29:23-34.
14. Kimani J, Maclean I, Bwayo J, et al. Risk factors for *Chlamydia trachomatis* pelvic inflammatory disease among sex workers in Nairobi, Kenya. *J. Infect. Dis.* 1996;173:1437-1444.
15. Conway D, Holland M, Bailey R, et al. Scarring trachoma is associated with polymorphism in the tumor necrosis factor alpha gene promoter and with elevated TNF-alpha levels in tear fluid. *Infect. Immun.* 1997;65:1003-1006.

16. West S, Munoz B, Lynch M, et al. Impact of face-washing on trachoma in Kongwa, Tanzania. *Lancet* 1995;345:155-58.
17. Thylefors B, Dawson C, Jones B, West S, Taylor H. A simple system for assessment of trachoma and its complications. *Bull WHO* 1987;65:477-483.
18. Gilroy C, Thomas B, Taylor-Robinson D. Small numbers of *Chlamydia trachomatis* elementary bodies on slides detected by the polymerase chain reaction. *J. Clin. Pathol.* 1992;45:531-532.
19. Bobo L, Coutlee F, Yolken R, Quinn T, Viscidi R. Diagnosis of *Chlamydia trachomatis* cervical infection by detection of amplified DNA with an enzyme immunoassay. *J. Clin. Microbiol.* 1990;28:1968-1973.
20. Bailey R, Duong T, Carpenter R. Age dependence of incidence, duration, and clinical features of trachoma episodes in hyperendemic areas. *Proc. Eur. Soc. Chlamydial Res.* 1996;3:177.
21. Mabey D, Bailey R, Ward M, Whittle H. A longitudinal study of trachoma in a Gambian village: implications concerning the pathogenesis of chlamydial infection. *Epidemiol. Infect.* 1992;108:343-351.
22. Sutter E, Ballard R. Community participation in the control of trachoma in Gazankulu. *Soc. Sci. Med* 1983;17:1813-1817.
23. Bailey R, Arullendran P, Whittle H, Mabey D. Randomized controlled trial of single-dose azithromycin in treatment of trachoma. *Lancet* 1993;342:453-456.
24. West S, Munoz B, Bobo L, et al. Nonocular chlamydial infection and risk of ocular reinfection after mass treatment in a trachoma hyperendemic area. *Invest Ophthalmol Vis Sci* 1993;34:3194-3198.
25. Beatty W, Byrne G, Morrison R. Morphologic and antigenic characterization of interferon-gamma mediated persistent *Chlamydia trachomatis* infection *invitro*. *Proc. Natl Acad Sci USA* 1993;90:3998-4001.
26. Hsieh Y-H, West S, Bobo L. DNA sequencing of *Chlamydia trachomatis* major outer membrane protein-1 gene indicates separate mechanisms of trachoma endemicity. (in press) 1998.
27. Bailey R, Downes B, Downes R, Mabey D. Trachoma and water use: a case control study in a Gambian village. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1991;85:824-828.
28. West S, Lynch M, Turner V, et al. Water availability and trachoma. *Bull World Health Organ* 1989;67:71-75.

Manuscript Two

DNA sequencing of *Chlamydia trachomatis* major outer membrane protein-1 gene indicates separate mechanisms of trachoma endemicity

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ABSTRACT

In a six year follow-up study of trachoma, DNA sequencing of *Chlamydia trachomatis* detected from 177 conjunctival samples revealed 26 *C. trachomatis* major outer membrane (*omp-1*) genotypes of serovar A and 18 of B/Ba. *Omp-1* genotypes evolved temporally, and only 20% of genotypes identified in 1995 were present in 1989. Strains with mutations in B-cell epitopes did not predominate or evolve as expected. However, selection of B/Ba genotypes exhibiting increased variable domain 2 amino acid substitutions occurred. Multitypic infection at baseline predicted subsequent severe disease and infection compared to monotypic infection, and was associated with familial cattle ownership. However, homotypic genotype infection with the same strain six years later was also associated with severe trachoma. Both evolution of *omp-1* genotypes, and sequential, homotypic infection are converging mechanisms of trachoma endemicity.

INTRODUCTION

Chlamydia trachomatis, the agent of trachoma, is still the leading cause of infectious blindness in many developing countries. It is thought that repeated episodes of infection contribute to conjunctival scarring, and the sequelae of trichiasis and blindness ¹. The immunodominant major outer membrane protein (*omp-1*) of *C. trachomatis* has been extensively studied, and its diversity was the basis of serologic classification of *C. trachomatis*. Nucleotide sequencing of the *omp-1* gene has shown that there are four variable domains (VD) I-IV which are interspaced with five highly conserved regions ². B and T-cell epitopes are present in VD I- IV in different serovars, and neutralizing antibodies to serovar and serogroup specific peptides can be generated ³⁻⁷. Short-term protective immunity against *C. trachomatis* has been demonstrated in animal studies and human trachoma vaccine trials ⁸⁻¹⁰. However, it is unclear why many individuals are not protected against subsequent natural infection.

It is thought that *omp-1* polymorphism contributes to *C. trachomatis* endemicity. Studies in high risk, sexually transmitted disease (STD) core groups in Kenya indicated that there was much diversity, and temporal change occurred in *omp-1* genotypes in genital infection ^{11, 12}. It has been postulated therefore that *C. trachomatis* variants with amino acid substitutions in B or T-cell epitopes could escape immune surveillance, and that these variants might contribute to *C. trachomatis* persistent infection and more severe diseases ¹³. In addition, both *in vitro* and human studies have shown that persistent infection may occur ^{14, 15}.

In this six-year survey of *C. trachomatis* conjunctival infection in trachoma hyper-endemic villages, both evolution of multiple *omp-1* genotypes and persisting infection with the same genotype suggest that separate host factors contribute to the lack of protective immunity and trachoma endemicity.

RESULTS

Overall C. trachomatis omp-1 sequence

Four serovars of *C. trachomatis* were identified in the 76% (177/233) of conjunctival samples that were positive by a diagnostic PCR for a conserved *omp-1* gene fragment. They included serovar A (63%), B/Ba (36%), C (0.6%) and E (1%). Nucleotide sequences of serovar C and E were identical to the reference strains, C/TW-3/OT and E/Bour. Sequence data are shown in Figure 1 for the 26 genotypes of serovar A, and 18 genotypes of serovar B/Ba. Since only six nucleotides differ between serovar B and Ba in VD I to IV, B/Ba was used to designate all serovar B and Ba-like local strains in which there were 2 to 4 nucleotide differences as compared to prototype B or Ba. There were also six genotypes of A and two of B/Ba that had only VD I and II sequence information. Genotype A was dominant in both 1989 (38%) and 1995 (40%). Genotypes B (1989:14%, 1995:18%) and B2d (1989:8%, 1995:12%) were also dominant, while the other genotypes were at low frequency and usually unique to a time point.

Unique or "signature" nucleotide (nt) substitutions were identified in all Tanzanian genotypes of serovar A and serovar B/Ba as compared to the reference strains A/Har-13, B/TW-5/OT and Ba/AP-2 that have been used for vaccine research (Figure 1). As indicated in Figure 1, four (80%) of five substitutions resulted in amino acid changes.

Nucleotide substitutions tended to occur at specific positions in the *omp-1* gene. Overall, 61% of nt substitutions from the study genotypes were the same at

corresponding position as compared to closely related serovars. For example, two of three signature nt substitutions of serovar A were also found in closely related serovar C: G → A, nt: 304, and A → C, nt: 523. Furthermore, 63%(39/62) nt substitution resulted in non-synonymous codons.

B-cell epitope mutations.

Two A genotypes, and five B/Ba genotypes detected from six (3%) different children (2 in 1989 and 4 in 1995) had amino acid substitution in known B-cell epitopes (Figure 1). However, there was trend for these strains not to become prevalent in either time point. *C. trachomatis* infection, disease status, and genotype at both time points are shown in Table 1. These genotypes did not predict disease severity, and genotypes were unique individually and temporally. As indicated, subject one lost the A4 genotype mutation in the B-cell epitope region at the second time point. Subject two evolved mutations from genotype A to A12b at the second time point, which indicated an endogenous evolution or an exogenous acquisition. Epidemiologic characteristics of children with B-cell epitope mutants were further examined. Five of the children were living in trachoma “high prevalence” villages, but there was no clustering of other epidemiologic variables as compared to other children.

Variable domain 2(VD-2) “hot spot” mutations

For serovar B/Ba, nucleotide substitutions occurred 2-3 times more frequently in the VD II region (14%) as compared to VD I and VD IV (7%), and VD III (5%). In addition, 50% of genotypes had single or multiple mutations in

VD II: nt 502-516 (Fig 1). Point mutations in 47% (7/15) occurred in this region, and 6/7 lead to amino acid substitutions. This region was designated a "hot spot", and there is overlap with a known B-cell epitope. For serovar A, the frequencies of nucleotide substitutions for VD I and VD II were 18% and 23%, respectively, while for VD III and VD IV they were 10%. No "hot spot" region was observed for serovar A.

Overall phylogenetic analyses

Genotype sequences were extremely close to each other. There were only 1 or 2 nucleotide differences between genotype A and other A genotypes. Similarly, there were only 1 to 3 nucleotide differences between genotype B and other B/Ba genotypes. Genotypes A1a and A14a formed one discrete clade within serovar A (Figure 2A). Three specific clades were identified within serovar B/Ba (Figure 2B). Genotypes B2f, B2d, B24b, B23a, B124a, B12a and B2c formed one subgroup, and B124a, B12a and Ba formed a cluster within this subgroup. The second and third clades were composed of B2a and B24a, B4c and B4d, respectively. In addition, B2a and B24a shared amino acid substitutions in a B cell epitope region, "¹³⁹NNENQTKVSN¹⁴⁸", of VD II (Figure 1).

Infection and disease in the same child in 1989 and 1995.

There were twenty-four children who had *C. trachomatis* infection at both time points, and genotyping was available for twenty-two (Table 2). Among them, 14% (3/22) had a serovar shift, and 41% (9/22) had a genotype shift. Moreover, 45% (10/22) had homotypic infection with the same genotype at both

time points. In terms of disease severity, 64% (14/22) had the same clinical grade at both time point. For the remaining 8 (36%), clinical status changed at the second time point; 6 went from severe inflammatory to follicular trachoma; and one from severe inflammatory trachoma to no sign of trachoma; and the other from follicular to severe inflammatory trachoma. For the 12 children who were available one year later in 1996, 11/12 had active trachoma and one had no disease.

There was no significant association between genotype shift and change of trachoma status. Multi-genotypic infection was common in this group of children, and >40% were infected with at least two genotypes simultaneously. Eight children had multiple but different genotypic infection at both time points. There were only 1 to 3 nucleotide differences between genotypes of the same serovar in the same individual cross-sectionally or temporally. In addition, eight children had monotypic infection in 1989 and in 1995.

Prediction of disease and infection by multitypic infection at a previous time point

Disease severity was not associated with a particular serovar or genotype. However, children with multitypic infection at a previous time point (1989 or 1995) were more likely in 1995 or 1996 to have severe inflammatory trachoma and infection (Table 3).

Epidemiologic factors and multitypic infection.

The association between multitypic infection and epidemiologic factors was examined in 49 children aged 1-2 years in 1989-90 and in 60 children aged 6-8 years in 1995. Children who were living in trachoma “high prevalence villages” in 1989-90 were more likely to have multitypic infection (O.R.= 11.05, 95% C.I. = 1.30-93.82, $p = 0.03$). In addition, familial cattle ownership in 1995 significantly increased the risk of having multitypic infection (O.R.= 4.36, 95% C.I.=1.16-16.37, $p=0.03$). In 1995, living in “high prevalence villages” did not predict for multitypic infection (O.R.=3.12, 95% C.I.= 0.62-15.75, $p=0.17$). Other epidemiologic factors, such as age, gender, maternal education, facial cleanliness, living in a house with a tin roof and water availability, were not significantly associated with multitypic infection.

Evolution, clinical severity and geographic cluster of “hot spot” genotypes.

Of the cohort of children aged 1-2 years in 1989, there were 14 and 20 children infected with serovars B/Ba in 1989 and 1995, respectively. “Hot spot” B/Ba genotypes became dominant in serovars B/Ba in 1995 in the cohort of children available for follow-up. There was a significant increase of “hot spot” mutations from 36% (5/14) in children aged 1-2 years in 1989 to 70% (14/20) in children aged 6-8 years in 1995 ($X^2=3.93$, $p=0.048$). However, the prevalence of “hot spot” genotypes in a second cohort of children 1-2 years old in 1995 was 32% (9/28). There was no association between disease severity and “hot spot”

genotypes. In addition, having a mutation in this region did not predict subsequent disease or infection in 1995 or 1996.

In order to study the geographic clustering of "hot spot" genotypes, a comparison was performed between "hot spot" and other B/Ba genotypes at the level of a balozi (a unit of ten houses). In 1995, 48% (23/48) of children who had "hot spot" genotypes were from eighteen balozis that were dispersed among five villages, while 23 (48%) children who did not have "hot spot" genotypes were from fifteen other balozis. There were only two children co-infected with "hot spot" and non-"hot spot" genotypes from two different villages. If only considering those balozis (10) where there were at least two children who were infected with serovar B/Ba, there was no co-existence of a "hot spot" genotype and other genotypes. Therefore, "hot spot" genovar infection was seen to cluster at the level of balozi.

Validation of omp-1 genotyping

Because of the genotype diversity observed in this study, a comparison of nucleotide sequences generated with Taq polymerase and high fidelity Pwo DNA polymerase in clones and original samples with unique substitutions was performed. Clones with nucleotide discrepancies by these two enzymes were retested by Pwo DNA polymerase, and the PCR products were sequenced to decide the final nucleotide sequences of the clones. Only 11 nucleotides (5.7×10^{-5} / nt / cycle) differed between these two enzymes in a total of 6378 nucleotides under comparison after 30 cycles of amplification. In addition,

nucleotide sequence data of the reference serovar A, B, and E strains were exactly the same as published data upon multiple sequencing following PCR from a stock of DNA.

All negative control samples were negative by *C. trachomatis* PCR.

DISCUSSION

In this study, the occurrence of multitypic and homotypic genotype infection at two time points in children living in trachoma-endemic villages suggests that separate but converging pathways could contribute to trachoma endemicity. Multitypic, but not monotypic infection, predicts subsequent infection and severe disease in children, and is associated with epidemiologic risk factors, such as familial cattle ownership. Extensive *omp-1* polymorphism, especially the evolution at a later time point of a hyper-substituted area of VD 2 in B/Ba strains, indicates that rapid genotypic evolution may contribute to ineffective host immunity. By contrast, infection and disease with the same strain at time points six years apart suggests that a separate immunologic mechanism could govern the inability to clear infection in this cohort.

Significant genetic diversity and evolution of *omp-1* VD 1 through 4 was observed in children living in hyper-endemic trachoma villages in this large study. A number of smaller studies in trachoma ¹⁵⁻¹⁸ and STD core populations ^{11-13, 19-21} have reported similar diversity. It has been hypothesized that the tempo of genetic change is more dynamic in endemic populations than in low prevalence settings due to increased exposure and pressure on the immune system ²². Indeed, genital isolates of serovars D and I with single point mutations escaped neutralization with monoclonal antibodies and human "immune" sera ²³. However, in a 3-month prospective trachoma study, chlamydia strains did not mutate in persistently or sporadically infected children from one village ¹⁵.

Genetic recombination has been documented between lymphogranuloma venereum strain, L1, and L2 isolated from STD patients in South Africa, that resulted in immunologically distinct strains ²⁴. The mechanism in terms of any interactions between the host immune system and chlamydial recombinant proteins has not been elucidated. In all other sequencing studies, point mutations have been documented ^{12, 13, 15-21}. In the present study, only point mutations were found, but serovar B/Ba-like genotypes were identified. Since only six nucleotides differed between the two types, these genotypes could not be classified and may thus represent a unique serovar. The predominant genotype B in this study differed from prototype B by two nucleotides, and from prototype Ba by four nucleotides, whereas study type B2d differed from either prototype B or Ba by three nucleotides. Interestingly, genotypes B and B2d were mutually exclusive in the ten-family, socio-geographic unit, the balozi. It is thus possible that study strains B and B2d also possess distinct immunotyping properties. Studies comparing these isolates with monoclonal antibodies, and human tears and sera are ongoing in our laboratory.

Increased mutation in a 15-nucleotide region of VD 2 of serovar B/Ba was observed in a cohort of children in 1989 in this study, and the resultant 10 "hot spot" genotypes evolved to predominate in 1995. Phylogenetic analysis indicated that they had a close relationship to prototype Ba serovar. For the second cohort of children in 1995, the "hot spot" strains were at the same level as the first cohort in 1989. In addition, only two "hot spot" strains could be identified in two

different individuals. This finding supports the view that natural selective pressures for dominance of these strains at the later time point could be related to host-immune pressure for mutation in an individual, and not to immigration of these strains from other villages. However, an alternative explanation is that “hot spot” genotype B2d was introduced to the susceptible cohort from other villages, while other “hot spot” strains were derived by host immune pressure.

In the present study, *omp-1* genotypes, A, B and B2d, were mostly responsible for observed trachoma hyper-endemicity. The other *omp-1* genotypes that had 1-3 nucleotide differences from the dominant genotypes were only identified in one subject at one time point. One possible explanation is that these strains mutated endogenously due to host immune selective pressure, but they could not compete with the other dominant genotypes either inside the host or during transmission to other hosts. Another possible explanation is that multitypic infection was due to exogenous re-infection with different genotypes during the study period. A third possibility is that these minor variants represent an artifact of PCR and/or sequencing, however, our control experiments suggest only a minority of variants would have been generated in this manner. In order to better understand the mechanism involved, prospective, sequencing studies at small time intervals would need to be performed.

A very small minority of children possessed strains with B-cell epitope mutations at both time points, and these genotypes were unique to a time point. Although five of the six children were from high trachoma prevalence villages,

high prevalence, per se, did not contribute to dominance of these strains. It could be argued that if these epitopes were in fact important in protective immunity, then mutation should have contributed to a concomitant rise in the prevalence of these strains.

It has been postulated that differences in disease severity in chlamydial infection may be due to strain-associated virulence properties. In studies of genital infection, F/G serovars were associated with less severe urethral symptoms in men ²⁵, whereas F genovariants were associated with more upper genital tract infection and pelvic inflammatory disease than E types in women ¹⁹. There have been no subsequent animal ocular or genital infection studies with these strains to further extend the observations of strain virulence. In our trachoma study, we did not find an association of particular chlamydial types with severe, inflammatory trachoma, either cross-sectionally or longitudinally. In addition, a 3-month longitudinal study in a trachoma-endemic village also did not find a particular type-association with infection duration ¹⁵. Our findings support the view that chlamydia virulence in trachoma may be determined by socio-environmental risk, and particularly, by the efficiency of host response to infection in a setting of high exposure and chlamydial *omp-1* mutation. For example, multitypic infection in 1989 predicted for continued infection and disease in 1995 and 1996 as compared to children who had monotypic infection. In addition, cattle-herding, as a risk factor, was associated with multitypic infection in 1995 for the cohort who were 1-2 year olds in 1989. This is an

illustration of the contribution of sociologic and behavioral differences to exposure between these age groups in the village. Younger children are more likely to be carried by their caregiver or to be inside the house, whereas older children tend cattle and play with each other. Although chlamydia has not been reported from ocular samples of cattle, the same genus of fluid-seeking flies, *Musca sorbens*, are also found near man and cattle. It is possible that flies may play a role in the association of cattle-herding and multitypic infection ^{26, 27}.

In the present study, there were also some children who had homotypic genotype infection at both time points, whether as monotypic or multitypic infection. These children maintained disease. In a previous study by our group, children were shown to be persistently infected for three months with the same strain ¹⁵. Therefore, some children cannot clear chlamydia infection for 3-month periods, and the present study suggests perhaps for as long as six years. Most likely, this cohort is different in regard to the immune mechanism that allows persisting infection or re-infection with the same strain compared to children with multitypic infection, or infection at only one time point. Comparison studies are ongoing in our laboratory to determine if there are differences in certain tear cytokines and antibodies in these children.

The efficacy of DNA sequencing studied by PCR can be affected by Taq polymerase nucleotide misincorporation. Comparison studies of high fidelity Pwo DNA polymerase and Taq validated that “false nucleotide mutations” were at a minimum. In addition, more than half of the nucleotide substitutions resulted

in non-synonymous codons, and signature nucleotide substitutions were identified all samples. This supports the view that Taq error was not a factor in the reported genotype diversity of this study.

The diversity and evolution of *omp-1* genotypes revealed in this study of trachoma endemic villages, and the concurrent finding of homotypic *omp-1* type infection at time points six years apart illustrates how multiple pathways contribute to trachoma endemicity. In under-served village populations, *omp-1* evolution due to immune pressure in an endemic setting should prompt researchers to choose another vaccine target. Moreover, in those children with "persisting" infection with the same strain, it will be important to determine if unique host factors distinguish them from their counterparts who cleared infection.

MATERIALS AND METHODS

Study population. Two groups of children from villages in the Kongwa district of central Tanzania were selected from a 1989 face-washing intervention trial. In the trial, two hundred and fifty eligible households in each village containing at least one child aged 1-7 years were randomly selected after a complete census. For our study, the first cohort, 289 children who were 1 or 2 years old in 1989-1990 from five villages in the Kongwa district, was identified from files of the face-washing study ²⁸; 176 were available for follow-up as 6-8 years old in 1995. In addition, 41 children in the first cohort who were part of a chronic trachoma case-control study were surveyed again in 1996. In order to better understand the trend of evolution of *omp-1* genotypes, a second cohort of 284 children aged 1-2 years old in 1995 were also sampled from the same villages and compared with 1-2 years old in 1989.

Epidemiologic characteristics including age, gender, residential villages, maternal education, familial cattle ownership, living in a house with tin roof, distance to water source, and child's facial cleanliness, were collected in 1989 and 1995 ²⁸. Subjects who had trachoma were treated with topical tetracycline after eye examination in accordance with WHO recommendations. This study was approved by the Joint Committee on Clinical Investigation of the Johns Hopkins Hospital in accordance with the Helsinki Declaration.

Trachoma grading and sample collection. Two photographs of the right tarsal conjunctivum of each participant were taken at all time points, and then graded by

trained readers with a 2.5X loupe using the simplified World Health Organization trachoma scheme ^{29, 30}. Clinical grading of trachoma was as follows: no signs of trachoma (NS); follicular trachoma if ≥ 5 follicles of ≥ 0.5 mm in diameter; and severe inflammatory trachoma (TI) with or without follicles if at least 50% of deep tarsal vessels were occluded. Active trachoma (TF) was defined as the presence of severe inflammatory trachoma or follicular trachoma.

In 1989, the right tarsal conjunctivum was sampled with a cotton-tipped dacron swab and fixed with acetone for direct fluorescent antibody (DFA) test. Slides were read using immunofluorescence microscopy for *omp-1* and then were frozen at -20 °C. The conjunctival samples collected in 1995 and 1996 were immediately placed in PCR holding buffer ³¹. All samples were collected after the photographs were taken.

Sample preparation (Appendix 2). Each DFA slide was scraped thoroughly and carefully with an individual razor blade, and the scrapings were suspended in 100 μ l of water and boiled for 10 minutes, after which a diagnostic PCR-EIA assay for a conserved *omp-1* gene fragment was performed ^{32, 33}. Processing controls for DNA contamination consisted of known DFA sample slides which were both DFA and PCR negative from a previous study, and were included every tenth sample slide ³¹. The 1995 samples, which were in transport buffer, were processed as described in a previous study ³¹.

PCR conditions (Appendix 2). A diagnostic PCR-EIA assay was performed on all samples using previously published methods ^{31, 32}. If samples were positive, a

1025 bp *omp-1* gene fragment was amplified using primers OMP-1 bp: 627 [5'-ACCACTTGGTGTGACGCTATCAG-3' sense] and OMP-2 bp: 1651 [5'-CTCACGTAAATGCACAATTCCG-3' antisense] which encompassed the four hypervariable regions. Next, a nested hypervariable PCR was performed to amplify a 937 bp *omp-1* gene fragment using primers OMP-N-1 bp: 653 [5'-GCGTATGGGTTACTATTGT-3' sense] and OMP-N-2 bp: 1589 [5'-GTGGATGCAGACAAATAC-3' antisense]. Amplified DNA was visualized using silver-stained polyacrylamide gels (Herring, 1982). Taq DNA polymerase (2.5 units) (Boehringer-Mannheim, Indianapolis, IN) was used in all PCRs.

SEQUENCING. The hypervariable *omp-1* nested PCR product was cloned according to the manufacturer's instructions (pCRTMII TA Cloning Kit, Invitrogen, San Diego, CA), and DNA from 3 transformants per sample were sequenced by the fluorescence dideoxy-terminator method of cycle sequencing (Applied Biosystems, Foster City, CA)(Smith, 1986; McCombie, 1992). The 937 bp DNA band was electro-eluted from a 0.8% agarose gel slice for sequencing. In order to control for sequencing accuracy, reference serovars A/Har-13, B/TW-5/OT and E/Bour (American Type Culture Collection, Rockville, MD) were sequenced multiple times. In addition, clones with specific nucleotide substitutions were re-sequenced from clones as well as original samples with high fidelity Pwo DNA polymerase (Boehringer-Mannheim, Indianapolis, IN) to compare sequence data with that generated using Taq polymerase.

GENOTYPING ANALYSIS. A unique omp-1 genotype was defined as

at least one nucleotide substitution in *omp-1* VD I to IV regions compared to the reference strains. A type-specific genotype nomenclature was devised and is illustrated by the example, A_{xyz}a. The first letter, A, would indicate the serovar type, the subscript(_x, _y or _z) indicates the variable domain containing the substitution compared to the dominant genotype (e.g., A). The last letter (a, b, etc.) would indicate the specific variant. The designation, dominant genotype, refers to the type which was most prevalent.

Nucleotide and amino acid sequences were aligned for each unique genotype as well as the reference strains A/Har-13, B/TW-5/ OT, and Ba/AP-2 using ESEE software (version 3.0s, Eric Cabot). Due to the occurrence of only 6 nucleotide differences in variable domains of *omp-1* gene between serovar B and serovar Ba, we designated those genotypes as serovar B/Ba because of the close relationship to B and Ba.

In order to understand whether selection of strains with substitutions in B cell epitope regions for prevalence in the study area occurred, sequences of genotypes were also compared to two known B cell epitope regions. They included “VAGLEK” at VD I of serovar A ³⁴ and “NNENQTKVSN” at VD II of serovar B ³⁵.

GenBank accession numbers (National Library of Medicine, Rockville, MD) were assigned sequentially for each variable domain of a given genotype from AF070227-AF070414 in the order of sequences in Figure 1. For example, genotype A, VD 1-4, would be AF070227, AF070228, AF070229 and AF070230,

and so on for each type when there was at least one nucleotide substitution.

PHYLOGENETIC ANALYSIS. In order to determine the topology of the phylogenetic tree, consensus values for the branch points on the phylogenetic tree were determined by generating 100 permuted trees using the SEQBOOT, DNADIST, and NEIGHBOR programs in the Phylogeny Inference Package (PHYLIP) package (version 3.5c.) distributed by Dr. Joseph Felsenstein. In addition, distance matrices for nucleotide and amino acid sequences were also calculated in order to determine the branch length of the phylogenetic tree. Finally, Fitch-Margoliash and least-squares distance methods in the PHYLIP package were used to determine the phylogenetic relatedness of the sequence set.

DATA ANALYSIS. Multitypic infection was defined if at least two different genotypes were simultaneously present in one ocular sample, while monotypic infection was defined if only one genotype was present. Two villages where the prevalence of trachoma was 77% each in 1989 were designated as trachoma "high prevalence villages". Low prevalence villages were the remainders, although trachoma rates were above 45%. Chi-square test or Fisher's Exact test was used to examine the association between disease severity and specific serovar, or genotypes at each time point. Logistic regression analysis was used to predict the likelihood of subsequent trachoma or *C. trachomatis* infection status in 1995 or 1996 by multitypic infection in 1989 or 1995, respectively. In addition, the association between epidemiologic variables and multitypic infection with *C. trachomatis* infection in 1989 or 1995 was examined by logistic

regression analysis. The results are expressed with odds ratios, 95% confidence intervals and two-tailed p-values. Statistical analyses were performed using SAS software (version 6.11, Cary, NC).

Table 1: *C. trachomatis* Infection, *omp-1* Genotypes, and Trachoma status in 1989 and 1995 in Children with Genotypes which had Substitution in Known B-cell Epitope Region

Subject	<i>C. trachomatis</i> ¹		Genotype		Disease Status ²	
	1989	1995	1989	1995	1989	1995
1	+	+	A4⁵, B2	A, A3	TI	TF
2	+	+	A	A12b	TI	TI
3	+	-	B2a, B24a	- o - ³	TF	TF
4	+	+	A, A4c	B, B1a, B2b	TI	TF
5	-	+	- o -	B12	TI	TI
6 ⁴	- o -	+	- o -	B2e	- o -	TI

¹ detected by PCR

² TI = severe inflammatory trachoma; TF = active trachoma

³ - o - = not available

⁴ aged 2 years old in 1995

⁵ Genotypes with substitutions in known B-cell epitope regions are in bold style

Table 2. Comparison of Disease Severity with Genotype in Children Available for Follow-up

Subject	<i>Genovar Type</i>		<i>Disease Status</i> ¹		
	1989	1995	1989	1995	1996
1	<u>A</u>	<u>A</u>	<u>TI</u>	<u>TI</u>	TI
2	<u>A</u>	<u>A</u>	<u>TI</u>	<u>TI</u>	- o - ²
3	<u>A</u>	<u>A</u>	TI	TF	TF
4	<u>A</u>	<u>A</u> , A2b	TI	TF	- o -
5	A12a, <u>A</u>	<u>A</u> , A3b	<u>TI</u>	<u>TI</u>	TF
6	A24a, A3c, <u>A</u>	<u>A</u>	<u>TI</u>	<u>TI</u>	- o -
7	A2	A	<u>TI</u>	<u>TI</u>	NS
8	A	A3	<u>TI</u>	<u>TI</u>	- o -
9	A	A3	TI	TF	- o -
10	A	A12b	<u>TI</u>	<u>TI</u>	- o -
11	A23a	A3a	TI	NS	TF
12	A3, A2c	A, A4e	TI	TF	TF
13	A1e, A1f, A4d	A	<u>TI</u>	<u>TI</u>	TI
14	B3a, <u>B</u>	<u>B</u>	<u>TI</u>	<u>TI</u>	TI
15	B4c, B4d, <u>B</u>	<u>B</u>	<u>TI</u>	<u>TI</u>	- o -
16	B3, <u>B</u>	<u>B</u> , B2c	<u>TF</u>	<u>TF</u>	- o -
17	B2f, <u>B2d</u>	<u>B2d</u> , B23a	<u>TI</u>	<u>TI</u>	TI
18	B,E	A	<u>TI</u>	<u>TI</u>	- o -
19	A, A4c	B, B1a, B2b	TI	TF	- o -
20	A4, B2	A, A3	TI	TF	TI
21	B2d, B24b	A, A2, A2a	<u>TI</u>	<u>TI</u>	TF
22	A, A4a	A1a, A3	TF	TI	TI

¹ TI= severe, inflammatory trachoma; TF= follicular; NS= no clinical sign of trachoma² - o - = Data not available

Table 3: Prediction of infection and disease status by multitypic *C. trachomatis* *omp-1* genotype infection at a previous time point

Category	Previous Infection Type (1989 or 1995)			p value
	Multitypic (N=18)	Monotypic (N=35)	O.R.(95% C.I.)	
Subsequent time point (1995 or 1996)				
Active Trachoma	16(89%)	25(70%)	3.20(0.62-16.54)	0.165
TI ¹	11(61%)	12(34%)	3.01(0.93-9.77)	0.066
<i>Chlamydia trachomatis</i> Infection ²	15(83%)	10(29%)	12.50(2.96-52.77)	0.001

Logistic regression analysis

¹TI = severe inflammatory trachoma

²infection status using PCR detection

FIGURE LEGENDS

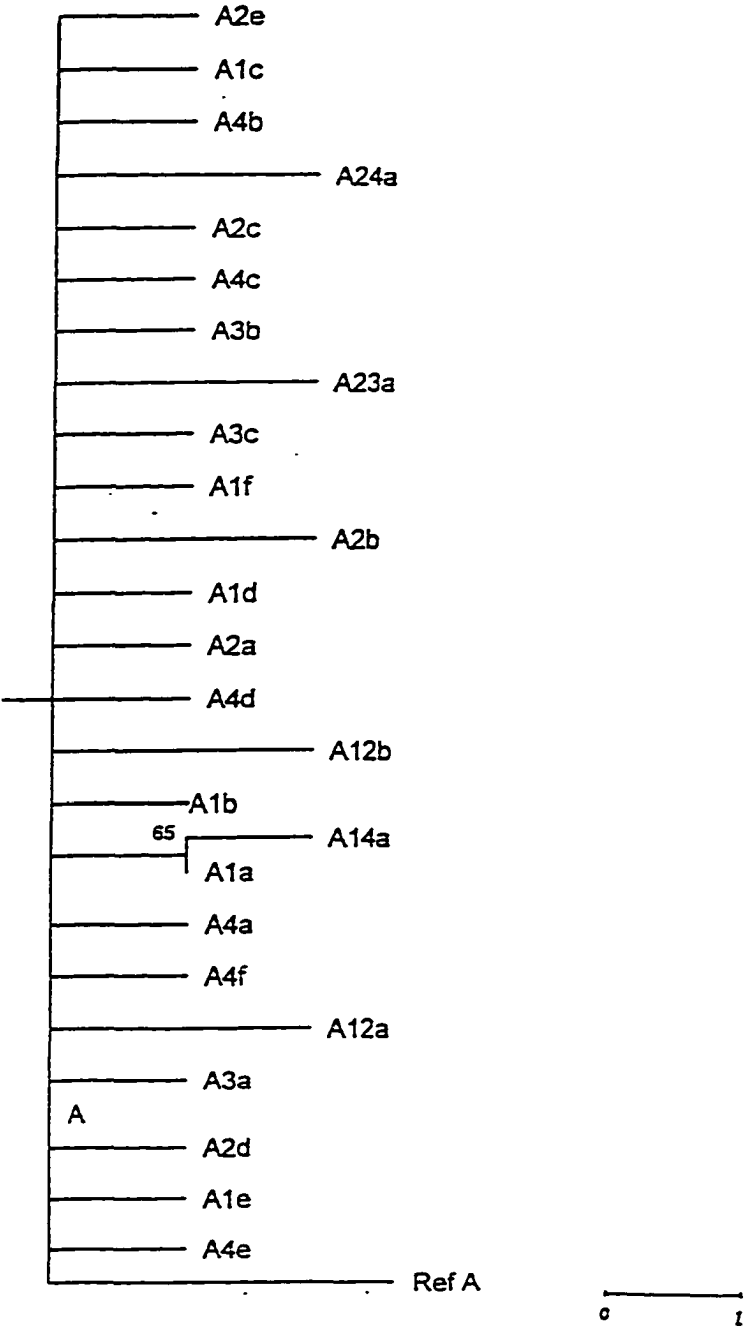
Figure 1. Nucleotide and deduced amino acid sequence for *omp-1* variable domain 1-4 of reference strain A/Har-13, B/TW-5/OT, and Ba/AP-2 and variant A and B/Ba genotypes. Dots indicate homology between reference and study strains; nucleotide substitution are indicated. Corresponding amino acids are listed above nucleotides, and substitutions in variants are indicated. * indicates B cell epitopes. ‡ indicates “hot spot” region for B/Ba genotypes.

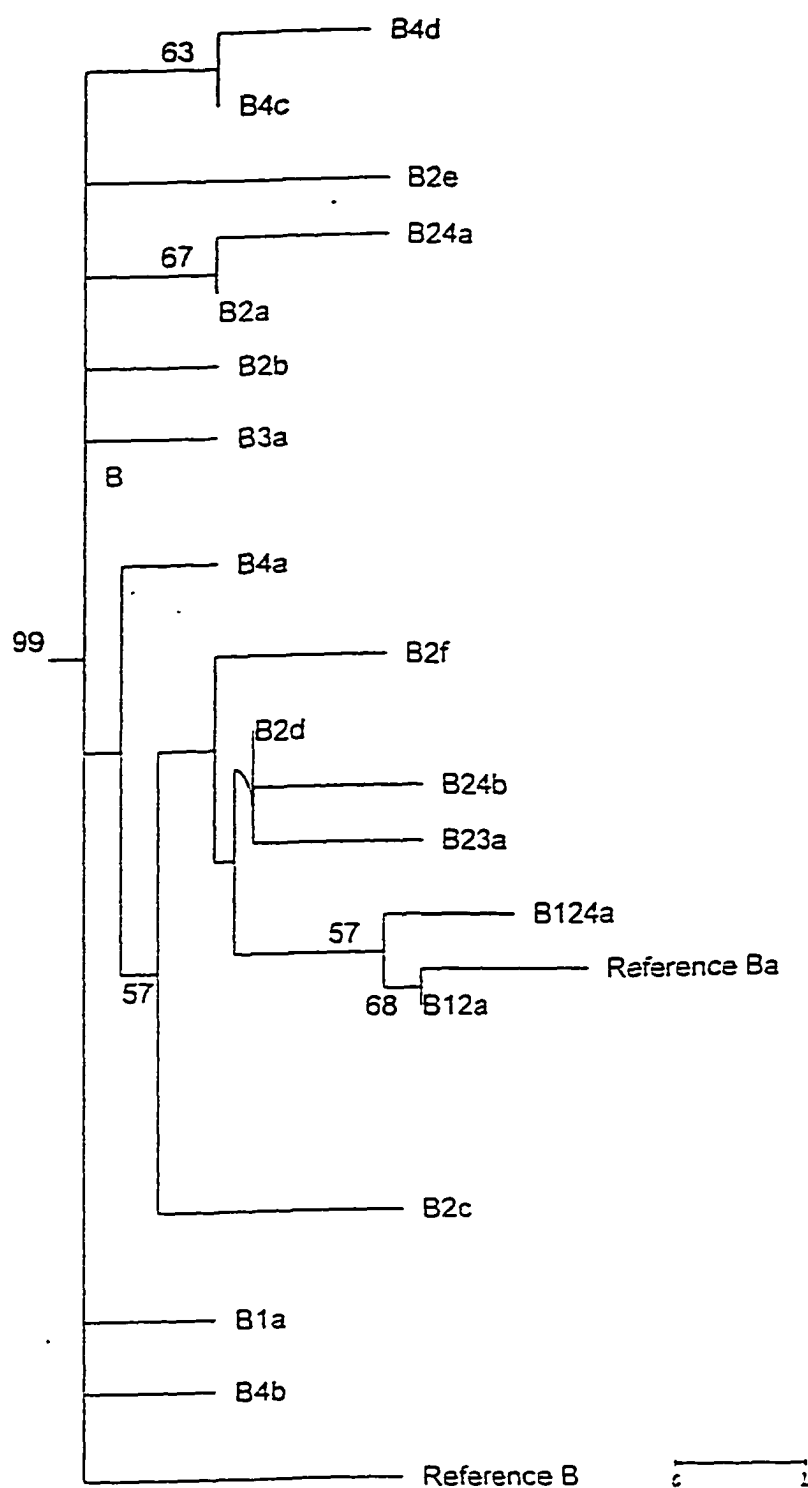
Serovar A	VP-3	VP-4
233	T A G T E A A	L A K P V L D T T T L N P T I A G K G T V V S S A E N E L A
234	ACCCGAGGAGAGAGCTGCG.....911	TTCGCTAACCAGAGCTTGATATACCACTACTCTAAACCCGACCAICCGTGTAAAGGACIGTGTCTCTCTGCGAGAAACGAACTGGCT
Study strains		
A		
A1a		
A1b		
A1c		
A1d		
A1e		
A1f		
A2a		
A2b		
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Figure 2A. Nucleotide phylogenetic tree of reference strain A/Har-13 and study variants A.

Figure 2B. Nucleotide phylogenetic tree of reference strain B/TW-5/OT and Ba/AP-2 and study variants B/Ba.

Bootstrap values greater than 50 between genotypes are shown at the branch points.





REFERENCES

1. Grayston, J., Wang, S.-P., Yeh, L.-J. & Kuo, C.-C. Importance of reinfection in the pathogenesis of Trachoma. *Rev Infect Dis* **7**, 717-725 (1985).
2. Yuan, Y., Zhang, Y., Watkins, N. & Caldwell, H. Nucleotide and deduced amino acid sequences for the four variable domains of the major outer membrane proteins of the 15 *Chlamydia trachomatis* serovars. *Infect. Immun.* **57**, 1040-1049 (1989).
3. Allen, J., Locksley, R. & Stephens, R. A single peptide from the major outer membrane protein of *Chlamydia trachomatis* elicits T cells help for the production of antibodies to protective determinants. *J. Immunol.* **147**, 674-679 (1991).
4. Su, H. & Caldwell, H. Immunogenicity of a chimeric peptide corresponding to T helper and B cell epitopes of the *Chlamydia trachomatis* major outer membrane protein. *J. Exp. Med.* **175**, 227-235 (1992).
5. Morrison, R., Manning, D. & Caldwell, H. Immunology of *Chlamydia trachomatis* infections: immunoprotective and immunopathogenetic responses. in *Sexually transmitted diseases* Vol. 8 (ed. Quinn, T.) 57-84 (Raven Press, Ltd., New York, 1992).
6. Villeneuve, A., Brossay, L., Paradis, G. & Hebert, J. Characterization of the humoral response induced by a synthetic peptide of the major outer membrane protein of *Chlamydia trachomatis* serovar B. *Infect. Immun.* **62**, 3547-3549 (1994).
7. Batteiger, B., Lin, P., Jones, R. & Van Der Pol, B. Species-, serogroup-, and serovar-specific epitopes are juxtaposed in variable sequence region 4 of the major outer membrane proteins of some *Chlamydia trachomatis* serovars. *Infect. Immun.* **64**, 2839-2841 (1996).
8. Wang, S.-P. & Grayston, J. Trachoma vaccine studies in monkeys. *Am. J. Ophthalmol.* **63**(Suppl.), 1615-1630 (1967).
9. Woolridge, R. *et al.* Long-term follow-up of the initial (1959-1960) trachoma vaccine field trial on Taiwan. *Am J Ophthalmol* **63**, 1650-1653 (1967).
10. Taylor, H. Ocular models of chlamydial infection. *Rev Infect Dis* **7**, 737-739 (1985).
11. Brunham, R. *et al.* *Chlamydia trachomatis* from individuals in a sexually transmitted disease core group exhibit frequent sequence variation in the major outer membrane protein (omp1) gene. *J. Clin. Invest.* **94**, 458-463 (1994).
12. Brunham, R. *et al.* The epidemiology of *Chlamydia trachomatis* within a sexual transmitted diseases core group. *J Infect Dis* **173**, 950-956 (1996).
13. Dean, D. & Millman, K. Molecular and mutation trends analyses of omp1 alleles for serovar E of *Chlamydia trachomatis*. *J. Clin. Invest.* **99**, 475-483 (1997).
14. Beatty, W., Morrison, R. & GI, B. Persistent Chlamydiae: from cell culture to a paradigm for chlamydial pathogenesis. *Microbiological Reviews* **58**, 686-699 (1994).

15. Bobo, L. *et al.* Severe disease in children with trachoma is associated with persistent *Chlamydia trachomatis* infection. *J Infect Dis* **176**, 1524-1530 (1997).
16. Dean, D., Schachter, J., Dawson, C. & Stephens, R. Comparison of the major outer membrane protein variant sequence regions of B/Ba isolates: a molecular epidemiologic approach to *Chlamydia trachomatis* infections. *J Infect Dis* **166**, 383-392 (1992).
17. Hayes, L. *et al.* Genotyping of *Chlamydia trachomatis* from a trachoma-endemic village in the Gambia by nested polymerase chain reaction: identification of strain variants. *J Infect Dis* **166**, 1173-1177 (1992).
18. Hayes, L. *et al.* Extent and kinetics of genetic change in the *omp1* gene of *Chlamydia trachomatis* in two villages with endemic trachoma. *J Infect Dis* **172**, 268-272 (1995).
19. Dean, D., Oudens, E., Bolan, G., Padian, N. & Schachter, J. Major outer membrane protein variants of *Chlamydia trachomatis* are associated with severe upper genital tract infections and histopathology in San Francisco. *J Infect Dis* **172**, 1013-1022 (1995).
20. Frost, E., Deslandes, S., D, G., Bourgaux-Ramoisy, D. & Bourgaux, P. Variation outside variable segments of the major outer membrane protein distinguishes trachoma from urogenital isolates of the same serovar of *Chlamydia trachomatis*. *Genitourin. Med.* **71**, 18-23 (1995).
21. Lampe, M., Wong, K. & Stamm, W. Sequence conservation in the major outer membrane protein gene among *Chlamydia trachomatis* strains from the upper and lower urogenital tract. *J. Infect. Dis.* **172**, 589-592 (1995).
22. Brunham, R., Plummer, F. & Stephens, R. Bacterial antigenic variation, host immune response, and pathogen-host coevolution. *Infect Immun* **61**, 2273-2276 (1993).
23. Lampe, M., Wong, K., Kuehl, L. & Stamm, W. *Chlamydia trachomatis* major outer membrane protein variants escape neutralization by both monoclonal antibodies and human immune sera. *Infect. Immun.* **65**, 317-319 (1997).
24. Hayes, L. *et al.* Evidence for naturally occurring recombination in the gene encoding the major outer membrane protein of lymphogranuloma venereum isolates of *Chlamydia trachomatis*. *Infect. Immun.* **62**, 5659-5663 (1994).
25. Van de Laar, M. *et al.* Differences in clinical manifestations of genital chlamydial infection related to serovars. *Genitourin Med* **72**, 261-265 (1996).
26. Taylor, H. *et al.* Hygiene factors and increased risk of trachoma in central Tanzania. *Arch Ophthalmol* **107**, 1821-1825 (1989).
27. Brechner, R., West, S. & Lynch, M. Trachoma and flies: Individual vs environmental risk factors. *Arch Ophthalmol* **110**, 687-689 (1992).
28. West, S. *et al.* Impact of face-washing on trachoma in Kongwa, Tanzania. *Lancet* **345**, 155-58 (1995).
29. Thylefors, B., Dawson, C., Jones, B., West, S. & Taylor, H. A simple system for assessment of trachoma and its complications. *Bull WHO* **65**, 477-483 (1987).

30. West, S. & Taylor, H. Reliability of photographs for grading trachoma in field studies. *Br J Ophthalmol* **74**, 12-13 (1990).
31. Bobo, L. *et al.* Diagnosis of *Chlamydia trachomatis* eye infection in Tanzania by polymerase chain reaction/enzyme immunoassay. *Lancet* **338**, 847-850 (1991).
32. Bobo, L., Coutlee, F., Yolken, R., Quinn, T. & Viscidi, R. Diagnosis of *Chlamydia trachomatis* cervical infection by detection of amplified DNA with an enzyme immunoassay. *J. Clin. Microbiol.* **28**, 1968-1973 (1990).
33. Gilroy, C., Thomas, B. & Taylor-Robinson, D. Small numbers of *Chlamydia trachomatis* elementary bodies on slides detected by the polymerase chain reaction. *J. Clin. Pathol.* **45**, 531-532 (1992).
34. Su, H. & Caldwell, H. Immunogenicity of a synthetic oligopeptide corresponding to antigenically common T-helper and B-cell neutralizing epitopes of the major outer membrane protein of *Chlamydia trachomatis*. *Vaccine* **11**, 1159-1166 (1993).
35. Su, H., Watkins, N., Zhang, Y.-X. & Caldwell, H. *Chlamydia trachomatis* host cell interactions: role of the chlamydial outer membrane protein as an adhesin. *Infect. Immun.* **58**, 1017-1025 (1990).

Comparisons of laboratory methods and trachoma clinical grading

PCR versus DFA (1986 & 1989, n=363)

Overall, 363 direct fluorescent antibody (DFA) samples, including 74 samples collected in 1986 and 289 samples collected in 1989, were processed and detected by *C. trachomatis* PCR. Seventy-five (89%) *C. trachomatis* DFA positive samples were *C. trachomatis* positive by PCR while 48 (17%) DFA negative samples were *C. trachomatis* positive by PCR (Appendix 3). The kappa value between these two assays was 0.62. There was a dose-response trend between these two assays by number of EBs on DFA, which was only available for the samples collected in 1986. The agreement between the two assays was 75% in samples containing 5-99 EBs while the agreement increased to 98% in samples containing over 100 EBs. In contrast, only 1 of 10 specimens that contained 1-4 EBs (defined as DFA negative), was positive by PCR. All negative control samples were negative by PCR.

PCR versus Clinical Grading of Trachoma

(a) DFA Slide Samples (1986 & 1989, n=363)

A pilot study was performed in order to apply *C. trachomatis* PCR on archival DFA slide samples. Seventy-four DFA slide samples collected from children aged 1-2 in Kongwa, Tanzania in 1986 and 289 DFA slide samples from children aged 1-2 in 1989 were tested in 1995.

Overall, 114 (45%) samples were positive by PCR from 254 children with active trachoma in 1986 and 1989. Among them, 60 (60%) children with severe

inflammatory trachoma were *C. trachomatis* positive by PCR. In addition, 11% of children (n=109) with no clinical signs of trachoma were positive by PCR (Appendix 3). The kappa value between clinical grading of trachoma and PCR on DFA slide samples was 0.25 for active trachoma and 0.33 for severe inflammatory trachoma.

(b) Samples in PCR Transport Buffer (1995, n=176)

Overall, 60 (62%) conjunctival swab samples from children (n=97) with active trachoma were positive by PCR. Among them, 36 (82%) children with severe inflammatory trachoma were positive by PCR. In addition, 10 (13%) children with no signs of active trachoma had *C. trachomatis* infection by PCR. The kappa value between clinical grading of trachoma and PCR on samples in transport buffer was 0.47 for active trachoma and 0.46 for severe inflammatory trachoma.

Photography Grading in Trachoma

The trained reader had high agreement with the trainer in the trachoma grading by photographs. A random sample of 20 pairs of photographs was used to compare the inter-observer variation. Only one difference in follicular trachoma (TF) and intense inflammatory trachoma (TI), respectively between the trained reader and the trainer. The kappa values for TF and TI were 0.90 and 0.88, respectively. In addition, 58 pairs of photographs which were difficult to grade by the reader were further examined by the reader and the trainer. There were 70% and 66% agreement between these two observers in TF and TI, respectively.

The kappa values for TF and TI were 0.38 and 0.35, respectively. The final trachoma grading for the 58 pairs of photographs was determined by the reader and the trainer together.

Sequencing

Serovar types of 9 samples determined by sequencing were compared to those by monoclonal antibodies in previous study (Taylor et al., 1989a). One previously identified as serovar A was identified as serovar B/Ba by sequencing. Other samples had the same serovar type identified as monoclonal antibody typing.

Sample Adequacy

DNA Adequacy of Sample

In order to assess the overall quality of DNA from archival DFA slides, we performed PCR for amplifying the conserved HLA-DRA and beta-globin gene fragments for 103 samples. Surprisingly, only 17% and 47% of samples were positive for HLA-DRA and beta-globin gene fragment, respectively (Appendix 4). All but two (12.5%) HLA-DRA positive samples also were beta-globin positive.

In further analysis, we found that positivity of *C. trachomatis* PCR but not DFA had differential effect on these two markers. There was no statistical difference in the result of HLA-DRA gene by *C. trachomatis* positivity (22.5% versus 14.3%, $p=0.285$) while there was a statistically significant difference for

beta-globin gene (65.5% versus 34.9%, $p=0.003$). In addition, there was a trend that the result of HLA-DRA or beta-globin gene fragment was determined by DFA result if PCR was negative. Noticeably, for PCR negative samples, there were relative lower positive rates for these two markers for DFA-positive samples when compared to those for DFA-negative samples (HLA-DRA: 0% for DFA(+) versus 16% for DFA(-), $p=0.580$; beta-globin: 14% for DFA(+) versus 38% for DFA(-), $p=0.405$). On the other hand, for PCR positive samples, prevalence of both markers was higher than PCR negative samples and there was no difference in prevalence for both markers by DFA result (HLA-DRA: 21% for DFA(-), 24% for DFA(+); beta-globin: 63% for DFA(-), 67% for DFA(+)). It indicated that the negative result of PCR from archival DFA-positive slides was determined by the adequacy and quality of sample DNA not the inhibitors in PCR.

Efficiency of HLA-DRA and beta-globin gene primers

In order to evaluate whether there was differential efficiency of both sets of primers, we also performed PCR for HLA-DRA and beta globin gene fragment on serial diluted known sheared human genomic DNA from Caucasians in 3 amplification. The data demonstrated that there were less than a 10-fold difference between these two sets of primers and the end point for both primer sets was the same at 200 fg/ μ l. Therefore, the data demonstrated that beta-globin gene was a better marker to assess the adequacy of DNA samples among Tanzanian children. Furthermore, unique HLA-DRA haplotype might be present and prevalent in this population.

Estimation of DNA Inadequate Samples

We can use information from DFA positive but both *C. trachomatis* PCR and beta globin gene PCR negative samples to estimate the prevalence of DNA inadequate samples. The percentage of negative result for *C. trachomatis* PCR and beta-globin gene PCR among DFA positive samples could be used as an index for DNA inadequacy. There were 21 PCR positive but beta-globin negative samples and one PCR negative but beta-globin positive samples in 28 DFA positive samples. Therefore, it was estimated that 61(21%) out of 289 samples in 1989-90 were inadequate for amplifying DNA. In addition, the prevalence of conserved HLA-DRA and beta-globin gene fragment after excluding DNA inadequate samples was 22% and 59%, respectively.

Results that are not presented in the manuscripts

Trachoma “high prevalence villages”

Two villages where the prevalence of active trachoma was 77% each were designated as trachoma-“high prevalence villages”. These two villages are very close too each other in proximity. In addition, over 90% of children in these two villages are one single ethnic group, Mkaguru. The other three villages, where the prevalence was relatively lower than that in “high prevalence villages”, were designated as trachoma “low prevalence villages”. In the low prevalence villages, only 46% of children are Mkaguru. In addition, “high prevalence villages” and

“low prevalence villages” were significantly different in familial cattle ownership, having a house with tin roof, and water availability (Appendix 5).

Univariate analysis for active trachoma in 1989

Crude odds ratios and 95% confidence intervals of risk factors by univariate analysis are shown in Appendix 6. Familial cattle ownership and living in “high prevalence villages” are associated with the presence of active trachoma in 1989.

Univariate analysis for severe inflammatory trachoma in 1989

Crude odds ratios and 95% confidence intervals of risk factors by univariate analysis are shown in Appendix 7. Familial cattle ownership, clean face, living greater than 2 hours away from a water source, and living in “high prevalence villages” are associated with the presence of severe inflammatory trachoma in 1989.

Univariate analysis for *C. trachomatis* infection in 1989

Crude odds ratios and 95% confidence intervals of risk factors by univariate analysis are shown in Appendix 8. Familial cattle ownership and living in “high prevalence villages” are associated with the presence of *C. trachomatis* in 1989. Having a clean face is protective against infection in 1989.

Univariate analysis for active trachoma in 176 follow-up children in 1995

Crude odds ratios and 95% confidence intervals of risk factors by univariate analysis are shown in Appendix 9. Living in “high prevalence villages” is associated with the presence of active trachoma in 1995.

Univariate analysis for severe inflammatory trachoma in 176 follow-up children in 1995

Crude odds ratios and 95% confidence intervals of risk factors by univariate analysis are shown in Appendix 10. Living in “high prevalence villages” is associated with the presence of severe inflammatory trachoma in 1995. In addition, being mkaguru marginally increased the risk of having severe inflammatory trachoma in 1995.

Univariate analysis for *C. trachomatis* infection in 176 follow-up children in 1995

Crude odds ratios and 95% confidence intervals of risk factors by univariate analysis are shown in Appendix 11. Living greater than 2 hours away from a water source, and living in “high prevalence villages” significantly increased the risk of having *C. trachomatis* infection in 1995, while having a clean face reduced the risk of having infection in 1995.

Variable “mother as caregiver”

In 1995, the follow-up children’s caregivers were identified as their mothers or others which included their grandmothers, aunts, other relatives or elder siblings. The data showed that children with their mothers as caregivers increased the risk of having active trachoma as compared to those with others as caregivers. This finding could be confounded by other underlying factors that we did not investigate in this study. It is more likely that children with their mothers

as caregivers had more younger siblings (under 5 years old) in their families as compared to children without their mothers as caregivers. It has been shown that children aged 2-3 years old were the high risk group for trachoma and having a sibling with trachoma was strongly associated with the presence of trachoma in this region. In addition, there was a differential distribution of mothers as caregiver in the “high prevalence villages” and “low prevalence villages”. Further investigation in the practice of mothers’ care of children in different ethnic groups is needed to understand the real impact of this variable.

Effects of clean face and distance to a water source greater than 2 hours on the risk of trachoma at both time points by familial cattle ownership status

In order to understand the interaction of familial cattle ownership and two other factors, clean face and distance to a water source on trachoma, a stratified analysis by familial cattle ownership was performed. The data showed that having an unclean face and distance to a water source greater than 2 hours increased the risk of having active trachoma at both time points for children without familial cattle ownership (Appendix). There were no effects for these two risk factors for children with familial ownership. In addition, there was no interaction for familial cattle ownership and clean face and distance to a water source on the presence of severe inflammatory trachoma at both time points.

Omp-1 genotypes in 1989 and 1995

Distribution of *omp-1* genotypes by serovars A and B/Ba and by years is shown in Appendices 12 and 13.

Amino acid phylogenetic tree of reference strains and study variants

Appendices 14 and 15 demonstrate the amino acid phylogenetic tree of reference strain A/Har-13 and study variants A in 1989-90 and 1995, respectively. Appendices 16 and 17 demonstrate the amino acid phylogenetic tree of reference strains B/TW-5/OT and Ba/AP-2 and study variants B/Ba in 1989-90 and 1995, respectively. Dominant genotype B2d formed a subgroup with reference strain Ba/AP-2 with bootstrap values greater than 50.

Limitations

The major concern of this study is the comparability of PCR results in samples 1989 and 1995. DNA adequacy for PCR in samples collected in 1989 was affected by the sample collection method, previous assay, 6-7 years of storage, and the preparation processing for PCR. DNA degradation was estimated in 25% of those samples by using PCR for HLA-DRA and beta-globin gene. Data also suggested that the impact of DNA degradation was more in those samples with lower chlamydial load as compared to those with higher chlamydial EB number. In addition, DNA degradation could also result in fragmented chlamydial DNA. Some samples with PCR(+) on the conserved *omp-1* gene fragment were not able to generate the whole *omp-1* gene fragment for sequencing. Therefore, the overall positive rate of *C. trachomatis* in 1989 was much lower. The number and type of *omp-1* genotype in 1989 were also affected, especially in children with mild diseases and infection. Risk factors for children with “persisting” infection at both time points cannot be identified. However, children with severe disease and infection in 1989 will not affect much. So, two separate mechanisms for trachoma endemicity based on 22 children with “persisting” infection” will be still valid. In addition, it will make our findings in *omp-1* polymorphism more strong if there were more unique genotypes in those DNA inadequate samples.

Another concern is that only two time points are in this six-year follow-up study. We do not know the number of episodes of disease or re-infection during the six-year period. In addition, we do not know when the risk factors changed and the impact of the change at that time on trachoma or infection. Our conclusions could be limited. Nevertheless, this study still provide significant information to researchers on trachoma epidemiology and *C. trachomatis* infection in a cohort of children followed for a long period of time.

Due to losses to follow-up and further stratification in data analysis, it is likely that we could not detect some true associations because of lack of power. Further investigation on epidemiologic variables with multitypic infection, and active trachoma with childhood mortality is needed to verify the associations.

Conclusions

Public Health Impact

The goals of this molecular epidemiologic study were to gain a better understanding of the interaction of epidemiologic and biologic factors with each other, and how these interactions contribute to trachoma endemicity in preschool children. The rationale for our study is what WHO recommendations of treating only symptomatic disease or mass treatment of a single village for purposes of trachoma control have failed. Secondly, a significant portion of chlamydia vaccine research has been directed at the immunodominant *omp-1* protein as a vaccine candidate, ignoring the possibility that mutation could render this antigen ineffect in an endemic region.

In this study, epidemiologic factors associated with “persisting” active trachoma and severe inflammatory trachoma at two time points six years apart in hyper-endemic villages were identified. Our data demonstrates that facial cleanliness reduces the risk of having severe disease even after six years of follow-up. The immediate goal of face-washing programs would be to decrease the prevalence of infection in children, with long-term benefit of lowering the incidence of trichiasis and blindness in adults. The incorporation of behavioral modification in local practices with cultural acceptance by the community will be an important factor in developing a societal intervention for trachoma at the community level. However, our study indicated that a substantial number of

children still have severe diseases at a later time point, and they maintain the same genotype of chlamydia at two time points six year apart. Therefore, a better understanding of the biologic factors related to the microorganism and its host are key in devising a combined strategy to control trachoma and blindness.

Biologic mechanisms of trachoma endemicity, that is, multitypic infection and homotypic *omp-1* genotype infection, were revealed in those children with “persisting” chlamydial infection. Extensive *omp-1* polymorphism, the tempo of mutation, and the absence of long term immunity against disease in this endemic region indicate that *omp-1* gene is not a good vaccine candidate. However, an efficacious vaccine remains desirable because of its cost-effectiveness in poor, under-served populations, especially in regions where water is scarce. There are two levels, involving the community and the individual that could substantially reduce *C. trachomatis* endemicity. Ostensibly, the tempo of *omp-1* mutation could slow down if the community burden of *C. trachomatis* was reduced, thus restricting infection to smaller and smaller socio-geographic units. As a result, *omp-1* mutation will decrease. At the individual level, reducing chlamydial load in the susceptible host could result in more rapid clearance of chlamydia with less chance of selection of *omp-1* variants by host immune pressure. Moreover, the chance of having persistent infection and disease could be reduced as the risk of having multitypic infection decreases. Finally, reduction of infectious burden will favorably impact on intra-familial or intra-balozi transmission of chlamydia.

This study also reveals a link between trachoma severity and childhood mortality, but further investigations are needed to verify the association and biologic cause. Nevertheless, this marker could provide a good and practical indicator for public health workers to identify high risk children in order to prevent early childhood mortality in endemic regions.

Future Direction in Trachoma Research

The B/Ba genotypes in this study may be a new serovar, because DNA sequencing indicated that they resembled both B and Ba. Tissue culture isolation and immunologic characterization of these genotypes are ongoing. Complete DNA sequencing on *omp-1* and immunotyping of culture isolates will determine if these genotypes are new serovars. Tear samples were also collected from these children because we were interested if the correlation of tear secretory IgA levels and Th1/Th2 cytokines with disease and infection could provide insight into the immunopathogenesis of *C. trachomatis* infection. In addition, we are collaborating with the CDC and other trachoma investigators worldwide to institute a better nomenclature for naming chlamydia genotypes, and to set-up a central sequencing database on the internet for researchers. This should facilitate both epidemiologic and immunologic research in trachoma..

The mechanism to why some individuals progress from trachomatous scarring to trichiasis remains unclear. To date, epidemiologic markers related to mothers as primary care-givers or cattle-ownership have been found to be associated with trichiasis in women. However, the association of cattle-

ownership has not been elucidated. In the present study, we found that familial cattle ownership was associated with multitypic infection, and that this is a predictor for persisting disease or infection. This result indicates that the cohort with multitypic infection could be at increased risk of trichiasis. However, studies on the basic host-related immuno-genetic mechanism related to development of trichiasis and the interaction with socio-environmental factors have not been performed. A better understanding of these factors could give additional insight on how to interrupt disease progression.

Some children in the present study had infection at two time points six years apart with a homotypic strain, and this suggests that persistent infection may occur in some hosts. It would be very important to determine if unique immuno-genetic characteristics distinguish these children from their counterparts who clear infection rapidly or who only have sporadic infection. Preliminary data in our laboratory indicate that some Tanzanian children have a point mutation in a highly conserved region of HLA-DRA-CD4 positive binding site. HLA typing of blood samples from Tanzanians correlated with infection persistence should also be investigated in order to determine if antigen presentation via particular HLA types is related to infection and disease variation. This information would be useful for vaccine research. In addition, it will be important to establish if persistent infection with the same genotype over longer time periods occurs, and if this could be a biologic factor contributing to the developing of trichiasis.

Research in trachoma may provide insight into the pathogenesis of genital chlamydial infections, and aid in development of a vaccine that could be useful in preventing genital infection and the sequelae of infection. Animal models can only contribute indirectly to immunologic and pathologic studies in humans. In addition, it is easier to follow the course of disease in trachoma than in human genital infection. It will be interesting to determine whether two different *omp-1* genotype infections contribute to the pathogenesis of genital chlamydial infection.

In conclusion, it could take a long time to eradicate the leading cause of infectious blindness in the world. Research in chlamydial pathogenesis, immunogenetics, and the interaction of *omp-1* polymorphism with epidemiologic factors are needed. Current findings suggest that a multifaceted approach will be needed in order to make a substantial public health impact in the control of infection and disease sequelae in poor and under-served populations of the world.

Appendix 1:

The Johns Hopkins University School of Medicine
The Johns Hopkins Hospital

Joint Committee on Clinical Investigation



Please address reply to:
Administration 129 / School of Medicine
720 Rutland Avenue / Baltimore MD 21205-2196

RPN NO : 95-06-02-05
Approved: 06/27/95



(410) 955-3003
FAX (410) 955-4267

June 27, 1995

Sheila K. West, PhD
Associate Professor, Ophthalmology
116 Wilmer
The Johns Hopkins Hospital

Dear Dr. West:

I am pleased to inform you that the Research Project Notification (RPN) entitled:

Molecular Epidemiological Study of Ocular C. Trachomatis Infection in a Trachoma Hyper-endemic Area (with Drs. Thomas Quinn, Linda Bobo, G. Mcallula, and Mr. Yu-Hsiang Hsieh) was reviewed and approved on June 27, 1995.

As Principal Investigator you are responsible for informing the listed coinvestigators of the status of the project. NO changes, amendments, or addenda may be made to the protocol without JCCI re-review and approval.

If adverse consequences or unexpected side effects are encountered in the course of the study, or if new information becomes available which could change the perception of a favorable risk:benefit ratio, you are responsible for informing the Committee PROMPTLY. The JCCI must review the new information to determine if the protocol should be modified, discontinued, or should continue as originally approved.

Accurate record keeping is extremely important. The JCCI makes periodic random audits to protocol records.

Approval is valid for one year. Renewal should be requested at least one month before approval expires. The reference number in the upper right corner has been assigned to the protocol, and we ask that you cite the number in future correspondence.

Sincerely yours,

Thomas R. Hendrix, M.D.
Chairman-JCCI
c/o 129 S/H Administration

TRH:cap
Enclosure
xc: P&T Committee
S/H CHR

Appendix 2: Laboratory Methods

Methods:

MOMP-Direct Fluorescent Antibody
slides from 1989 samples

1995 samples collected in
PCR transport buffer

↓
scrape and boil

diagnostic PCR-EIA for conserved omp-1 (280bp)

↓
positive

↓
whole omp-1 PCR (1038bp) & nested PCR (937bp)

↓
cloning/sequencing omp-1 VD I-IV for all samples

↓
sequence comparison to reference strains using ESEE3S Software

Appendix 3: Comparison of *C. trachomatis* PCR from archival DFA¹ slides and DFA test and clinical trachoma in children in 1986 and 1989, Kongwa Tanzania

Category	Total No.	<i>C. trachomatis</i> (+) by PCR	
		No.	(%)
A. DFA Test ¹			
<i>C. trachomatis</i> (-)	279	48	(17%)
<i>C. trachomatis</i> (+) ²	84	75	(89%)
B. Clinical Trachoma			
No Sign	109	12	(11%)
Active Trachoma	254	114	(45%)
Severe Inflammatory Trachoma	100	60	(60%)

¹ direct fluorescent antibody against *C. trachomatis* major outer membrane protein-1

² with ≥ 5 elementary bodies

Appendix 4: Amplification of conserved HLA-DRA gene and β -globin gene fragments on DFA slides samples from children in 1986 & 1989 by *C. trachomatis* DFA¹ and PCR status

<i>C. trachomatis</i> Infection	Total No.	HLA-DRA(+)	β -globin(+)
		No.(%)	No.(%)
PCR(-) and DFA(-)	56	9(16%)	21(38%)
PCR(-) and DFA(+) ²	7	0 (0%)	1(14%)
PCR(+) and DFA(-)	19	4(21%)	12(63%)
PCR(+) and DFA(+)	21	5(24%)	14(67%)
Total	103	18(17%)	48(47%)

¹ direct fluorescent antibody against *C. trachomatis* major outer membrane protein-1

² with ≥ 5 elementary bodies

Appendix 5: Distribution of characteristics in 289 children aged 1-2 years old in trachoma “high prevalence villages”¹ and “low prevalence villages” in 1989

Characteristics	High Prevalence Villages		Low Prevalence Villages	
	No.	%	No.	%
Age (2 years)	77	57.0	91	59.1
Gender(Female)	73	54.1	76	49.4
Ethnic Group(Mkaguru) ^{2*}	78	90.7	41	45.6
Cattle Herding*	60	44.4	24	15.6
Tin Roof*	21	15.6	46	30.1
Clean Face	79	58.5	89	57.8
Water > 2 Hours*	97	71.9	25	16.2
Maternal Education	67	49.6	80	51.9

* $p < 0.05$, chi-square test

¹ High prevalence villages were referred to two villages where the prevalence of active trachoma was 77% each in 1989. The remainder was designated as “low prevalence villages”, although trachoma rates were between 45% to 65%.

² Data only available in 176 follow-up children in 1995

Appendix 6: Association of characteristic and active trachoma in 1989 in 289 children aged 1-2 in 1989 by univariate analysis

Characteristics	Active Trachoma in 1989		
	Odds Ratio	95% C.I.	p-value
Age (2 years versus 1 year)	0.82	0.50-1.36	0.4455
Gender(Female)	0.86	0.53-1.40	0.5386
High Prevalence Villages ¹	2.58	1.55-4.31	0.0003
Cattle Ownership	3.87	2.01-7.44	0.0001
Tin Roof	0.81	0.46-1.44	0.4730
Clean Face	0.64	0.38-1.05	0.0775
Water >2 Hours	1.51	0.91-2.49	0.1099
Maternal Education	0.77	0.47-1.26	0.3025

Logistic regression analysis

¹ High prevalence villages were referred to two villages where the prevalence of active trachoma was 77% each in 1989. The remainder was designated as “low prevalence villages”, although trachoma rates were between 45% to 65%.

Appendix 7: Association of characteristic and severe inflammatory trachoma in 1989 in 289 children aged 1-2 in 1989 by univariate analysis

Characteristic s	Severe Inflammatory Trachoma in 1989		
	Odds Ratio	95% C.I.	p-value
Age (2 years versus 1 year)	0.58	0.35-0.97	0.0380
Gender(Female)	0.80	0.48-1.33	0.3903
High Prevalence Villages ¹	2.37	1.41-3.98	0.0011
Cattle Ownership	2.70	1.58-4.63	0.0003
Tin Roof	0.91	0.50-1.66	0.7590
Clean Face	0.39	0.23-0.65	0.0003
Water >2 Hours	2.06	1.23-3.43	0.0057
Maternal Education	0.78	0.47-1.29	0.3357

Logistic regression analysis

¹ High prevalence villages were referred to two villages where the prevalence of active trachoma was 77% each in 1989. The remainder was designated as “low prevalence villages”, although trachoma rates were between 45% to 65%.

Appendix 8: Association of characteristic and *C. trachomatis* infection in 1989 in 289 children aged 1-2 in 1989 by univariate analysis

Characteristics	<i>C. trachomatis</i> infection in 1989		
	Odds Ratio	95% C.I.	p-value
Age (2 years versus 1 year)	1.09	0.62-1.90	0.7663
Gender(Female)	0.65	0.37-1.13	0.1234
High Prevalence Villages ¹	2.33	1.33-4.09	0.0032
Cattle Ownership	2.32	1.31-4.10	0.0039
Tin Roof	0.85	0.43-1.63	0.6007
Clean Face	0.28	0.16-0.49	0.0001
Water >2 Hours	1.70	0.98-2.94	0.0594
Maternal Education	1.07	0.62-1.86	0.7975

Logistic regression analysis

¹ High prevalence villages were referred to two villages where the prevalence of active trachoma was 77% each in 1989. The remainder was designated as “low prevalence villages”, although trachoma rates were between 45% to 65%.

Appendix 9: Association of characteristic and active trachoma in 1995 in 176 follow-up children in 1995 by univariate analysis

Characteristics	Active Trachoma in 1995		
	Odds Ratio	95% C.I.	p-value
Age (2 years versus 1 year in 1989)	0.99	0.54-1.83	0.9816
Gender(Female)	0.70	0.38-1.27	0.2348
Ethnic Group(Mkaguru)	1.43	0.76-2.70	0.2696
High Prevalence Villages ¹	2.71	1.47-5.01	0.0015
Cattle Ownership(1995)	1.64	0.87-3.08	0.1267
Tin Roof(1995)	1.68	0.83-3.38	0.1467
Clean Face(1995)	0.83	0.37-1.87	0.6559
Water >2 Hours(1995)	1.82	0.88-3.77	0.1084
Maternal Education	1.45	0.80-2.64	0.2201

Logistic regression analysis

¹ High prevalence villages were referred to two villages where the prevalence of active trachoma was 77% each in 1989. The remainder was designated as “low prevalence villages”, although trachoma rates were between 45% to 65%.

Appendix 10: Association of characteristic and severe inflammatory trachoma in 1995 in 176 follow-up children in 1995 by univariate analysis

Characteristics	Severe Inflammatory Trachoma in 1995		
	Odds Ratio	95% C.I.	p-value
Age (2 years versus 1 year in 1989)	0.77	0.38-1.54	0.4614
Gender(Female)	0.88	0.45-1.75	0.7201
Ethnic Group(Mkaguru)	2.20	0.98-4.98	0.0577
High Prevalence Villages ¹	3.36	1.61-7.00	0.0013
Cattle Ownership(1995)	1.75	0.87-3.51	0.1176
Tin Roof(1995)	1.10	0.51-2.39	0.8048
Clean Face(1995)	0.59	0.25-1.38	0.2234
Water >2 Hours(1995)	1.58	0.73-3.42	0.2474
Maternal Education	1.00	0.51-1.98	1.0000

Logistic regression analysis

¹ High prevalence villages were referred to two villages where the prevalence of active trachoma was 77% each in 1989. The remainder was designated as “low prevalence villages”, although trachoma rates were between 45% to 65%.

Appendix 11: Association of characteristic and *C. trachomatis* infection in 1995 in 176 follow-up children in 1995 by univariate analysis

Characteristics	<i>C. trachomatis</i> infection in 1995		
	Odds Ratio	95% C.I.	p-value
Age (2 years versus 1 year in 1989)	1.18	0.63-2.21	0.6014
Gender(Female)	0.81	0.44-1.49	0.5000
Ethnic Group(Mkaguru)	1.68	0.86-3.27	0.1259
High Prevalence Villages ¹	2.85	1.53-5.34	0.0010
Cattle Ownership(1995)	1.41	0.75-2.65	0.2822
Tin Roof(1995)	0.89	0.45-1.79	0.7514
Clean Face(1995)	0.40	0.18-0.91	0.0280
Water >2 Hours(1995)	2.08	1.02-4.21	0.0434
Maternal Education	0.94	0.52-1.73	0.8528

Logistic regression analysis

¹ High prevalence villages were referred to two villages where the prevalence of active trachoma was 77% each in 1989. The remainder was designated as “low prevalence villages”, although trachoma rates were between 45% to 65%.

Appendix 12: Effects of clean face and distance to water on risk of having active trachoma at both time points six years apart by familial cattle ownership status, Kongwa, Tanzania, 1989-1995

Characteristics	Category	Cattle Ownership		No Cattle Ownership	
		No.	Trachoma ¹	No.	Trachoma
Clean Face	No	20	13(65%)	72	32(44%)
	Yes	23	15(65%)	60	17(28%) ²
Distance to Water	< 2 hours	29	17(59%)	114	39(34%)
	>= 2 hours	15	12(80%)	17	10(59%) ³

¹ Active trachoma at both time points

² p=0.056

³ p=0.050

Appendix 13: Distribution of *omp-I* genotypes A in 1989 and 1995

Genotype	Frequency(%)	
	1989 (n=43)	1995(n=85)
A	19(44)	51(60)
A12a	1 (2)	0
A1a	0	1 (1)
A14a	0	1 (1)
A12b	0	1 (1)
A1b	1 (2)	3 (4)
A1c	1 (2)	0
A1d	1 (2)	0
A1e	1 (2)	0
A1f	1 (2)	0
A2a	0	1 (1)
A2b	0	1 (1)
A23a	1 (2)	0
A2c	1 (2)	0
A24a	1 (2)	0
A2d	0	1 (1)
A2e	1 (2)	0
A3a	0	1 (1)
A3b	0	1 (1)
A3c	1 (2)	0
A4a	0	1 (1)
A4b	0	1 (1)
A4c	1 (2)	0
A4d	1 (2)	0
A4e	0	1 (1)
A4f	1 (2)	1 (1)
A2 ¹	1 (2)	1 (1)
A3 ²	6(14)	12(14)
A4 ¹	1 (2)	0
A9 ²	0	1 (1)
A10 ²	1 (2)	2 (2)
A12 ²	0	1 (1)
A25 ²	0	1 (1)
A26 ²	0	1 (1)
A28 ²	1 (2)	0

¹ only have VD I sequence information² only have VD I and II sequence information

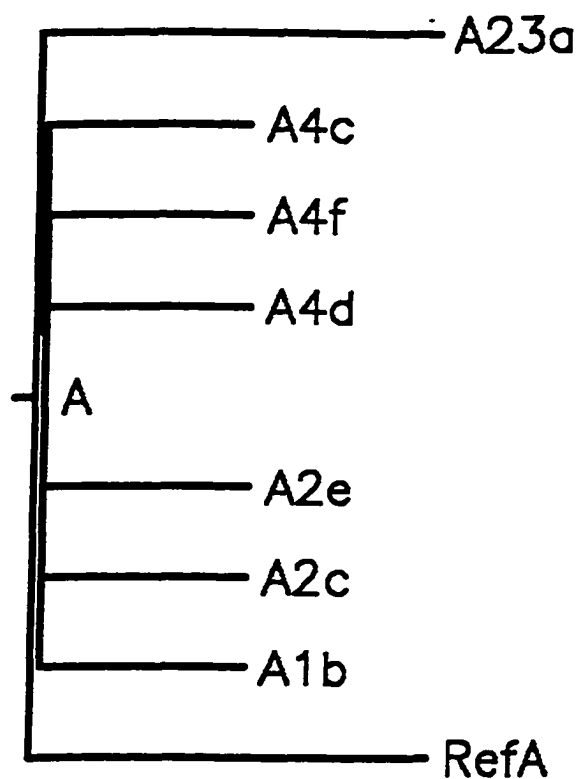
Appendix 14: Distribution of *omp-I* genotypes B/Ba in 1989 and 1995

Genotype	Frequency(%)	
	1989 (n=21)	1995(n=53)
B	7(33)	23(43)
B1a	0	1 (2)
B12a	0	2 (4)
B124a	0	1 (2)
B2a	1 (5)	0
B24a	1 (5)	0
B2b	0	1 (2)
B2c	0	1 (2)
B2d	4 (19)	15(35)
B24b	1 (5)	0
B23a	0	1 (2)
B2e	0	1 (2)
B2f	1 (5)	0
B3a	1 (5)	0
B4a	1 (5)	0
B4b	0	1 (2)
B4c	1 (5)	0
B4d	1 (5)	0
B2 ¹	1 (5)	0
B3 ²	1 (5)	2 (4)
B10 ²	0	1 (2)
B12 ²	0	1 (2)
B13 ²	0	2 (4)

¹ only have VD I sequence information² only have VD I and II sequence information

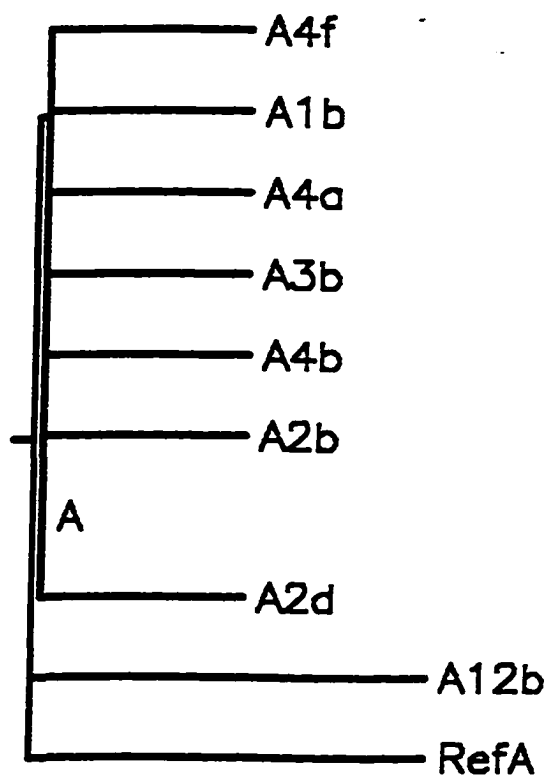
Appendix 15 :

Amino acid phylogenetic tree of reference strain A/Har-13 and study variants A in 1989



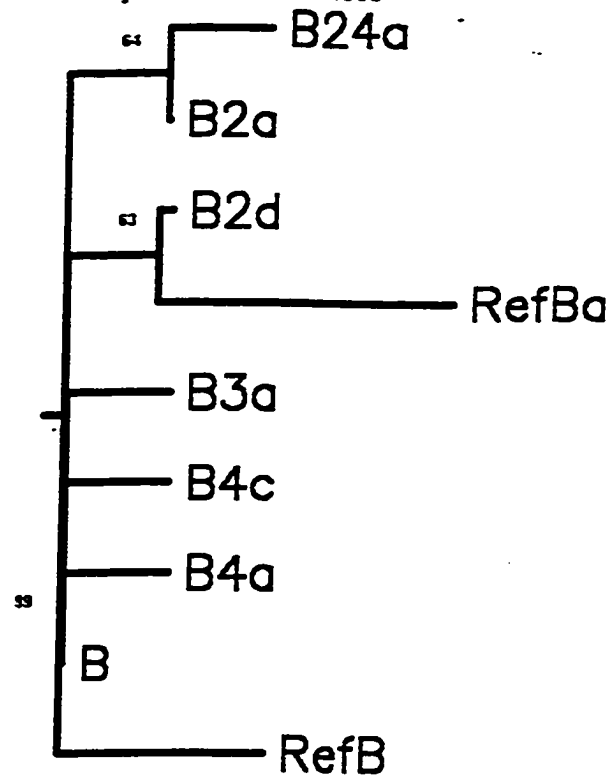
Appendix 16 :

Amino acid phylogenetic tree of reference strain A/Har-13 and study variants A in 1995



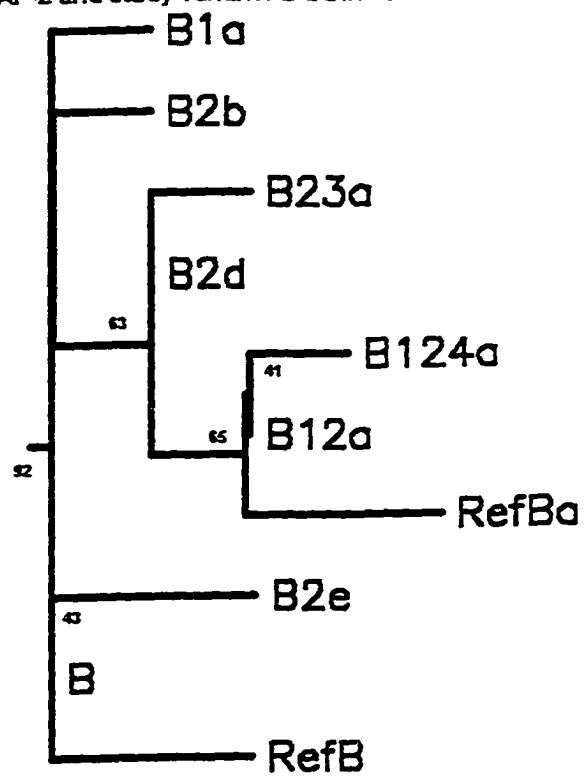
Appendix 17 :

Amino acid phylogenetic tree of reference B/TW-5/OT and
Ba/AP-2 and study variants B/Ba in 1989



Appendix 18 :

Amino acid phylogenetic tree of reference strain B/TW-5/OT and Ba/AP-2 and study variants B/Ba in 1995



Bibliography

- Allen, J., Locksley, R. and Stephens, R.: A single peptide from the major outer membrane protein of *Chlamydia trachomatis* elicits T cells help for the production of antibodies to protective determinants. J. Immunol. 147 (1991) 674-679.
- Aronson, S.: Trachoma and immigration. Medicine & Health, Rhode Island 80 (1997) 76-7.
- Bailey, R., Arullendran, P., Whittle, H. and Mabey, D.: Randomized controlled trial of single-dose azithromycin in treatment of trachoma. Lancet 342 (1993a) 453-456.
- Bailey, R., Downes, B., Downes, R. and Mabey, D.: Trachoma and water use; a case control study in a Gambian village. Transactions of the Royal Society of Tropical Medicine and Hygiene 85 (1991) 824-828.
- Bailey, R., Duong, T. and Carpenter, R.: Age dependence of incidence, duration, and clinical features of trachoma episodes in hyperendemic areas. Proc. Eur. Soc. Chlamydial Res. 3 (1996) 177.
- Bailey, R., Hayes, L., Pickett, M., Whittle, H., Ward, M. and Mabey, D.: Molecular epidemiology of trachoma in a Gambian village. Br J Ophthalmol 78 (1994) 813-817.
- Bailey, R., Kajbaf, M., Whittle, H., Ward, M. and Mabey, D.: The influence of local antichlamydial antibody on the acquisition and persistence of human

ocular chlamydial infection: IgG antibodies are not protective. *Epidemiol. Infect.* 111 (1993b) 315-324.

Bailey, R., Osmond, C., Mabey, D., Whittle, H. and Ward, M.: Analysis of the household distribution of trachoma in a Gambian village using Monte Carlo simulation procedure. *Int J Epidemiol* 18 (1989) 944-951.

Batteiger, B.: The major outer membrane protein of a single *Chlamydia trachomatis* serovar can possess more than one serovar-specific epitope. *Infect. Immun.* 64 (1996) 542-547.

Batteiger, B., Lin, P., Jones, R. and Van Der Pol, B.: Species-, serogroup-, and serovar-specific epitopes are juxtaposed in variable sequence region 4 of the major outer membrane proteins of some *Chlamydia trachomatis* serovars. *Infect. Immun.* 64 (1996) 2839-2841.

Batteiger, B., Rank, R., Bavoil, P. and Soderberg, L.: Partial protection against genital reinfection by immunization of guinea-pigs with isolated outer-membrane proteins of the chlamydial agent of guinea-pig inclusion conjunctivitis. *J. Gen. Microbiol.* 139 (1993) 2965-2972.

Beatty, P. and Stephens, R.: CD8+ T lymphocyte-mediated lysis of *Chlamydia*-infected L cells using an endogenous antigen pathway. *J. Immunol.* 153 (1994) 4588-4595.

Beatty, W., Morrison, R. and GI, B.: Persistent *Chlamydiae*: from cell culture to a paradigm for chlamydial pathogenesis. *Microbiological Reviews* 58 (1994) 686-699.

- Black, C.: Current methods of laboratory diagnosis of *Chlamydia trachomatis* infections. Clin Microbiol Rev 10 (1997) 160-184.
- Bobo, L., Munoz, B., Viscidi, R., Quinn, T., Mkocho, H. and West, S.: Diagnosis of *Chlamydia trachomatis* eye infection in Tanzania by polymerase chain reaction/enzyme immunoassay. Lancet 338 (1991) 847-850.
- Bobo, L., Novak, N., Mkocho, H., Vitale, S., West, S. and Quinn, T.: Evidence for a predominant proinflammatory conjunctival cytokine response in individuals with trachoma. Infect. Immun. 64 (1996) 3273-3279.
- Bobo, L., Novak, N., Munoz, B., Hsieh, Y., Quinn, T. and West, S.: Severe disease in children with trachoma is associated with persistent *Chlamydia trachomatis* infection. J Infect Dis 176 (1997) 1524-1530.
- Brunham, R.: Vaccine design for the prevention of *Chlamydia trachomatis* infection. In: Orfila, J., Byrne, G., Chernesky, M., Grayston, J., Jones, R., Ridgway, G., Saikku, P., Schachter, J., Stamm, W. and Stephens, R. (Eds.), Chlamydial infections - proceedings of the Eighth International Symposium on Human Chlamydial Infections. Societa Editrice Esculapio, Bologna, Italy, 1994, pp. 73-82.
- Brunham, R., Kimani, J., Bwayo, J., Maitha, G., Maclean, I., Yang, C., Shen, C., Roman, S., Nagelkerke, N., Cheang, M. and Plummer, F.: The epidemiology of *Chlamydia trachomatis* within a sexual transmitted diseases core group. J Infect Dis 173 (1996) 950-956.

Brunham, R., Yang, C., Maclean, I., Kimani, J., Maitha, G. and Plummer, F.:

Chlamydia trachomatis from individuals in a sexually transmitted disease core group exhibit frequent sequence variation in the major outer membrane protein (omp1) gene. J. Clin. Invest. 94 (1994) 458-463.

Caldwell, H. and Perry, L.: Neutralization of *Chlamydia trachomatis* infectivity with antibodies to major outer membrane protein. Infect Immun 38 (1982) 745-754.

Congdon, N., West, S., Vitale, S., Ktala, S. and Mmbaga, B.: Exposure to children and risk of active trachoma in Tanzanian women. Am. J. Epidemiol. 137 (1993) 366-372.

Conway, D., Holland, M., Bailey, R., Campbell, A., Mahdi, O., Jennings, R., Mbeni, E. and Mabey, D.: Scarring trachoma is associated with polymorphism in the tumor necrosis factor alpha gene promoter and with elevated TNF-alpha levels in tear fluid. Infect. Immun. 65 (1997) 1003-1006.

Conway, D., Holland, M., Campbell, A., Bailey, R., Krausa, P., Peeling, R., Whittle, H. and Mabey, D.: HLA class I and II polymorphisms and trachomatous scarring in a *Chlamydia trachomatis*- endemic population. J Infect Dis 174 (1996) 643-6.

Cotter, T. and Byrne, G.: Immunity to Chlamydia: comparison of human infections and murine models. Forum in Immunology (1996) 587-595.

- Courtright, P., Sheppard, J., Schachter, J., Said, M. and Dawson, C.: Trachoma and blindness in the Nile Delta: current patterns and projections for the future in the rural Egyptian population. *Br. J. Ophthalmol.* 73 (1989) 536-540.
- Dawson, C., Jones, B. and Tarizzo, M.: Guide to trachoma control. World Health Organization, Geneva, Switzerland, 1981.
- Dean, D. and Millman, K.: Molecular and mutation trends analyses of *omp1* alleles for serovar E of *chlamydia trachomatis*. *J. Clin. Invest.* 99 (1997) 475-483.
- Dean, D., Oudens, E., Bolan, G., Padian, N. and Schachter, J.: Major outer membrane protein variants of *Chlamydia trachomatis* are associated with severe upper genital tract infections and histopathology in San Francisco. *J Infect Dis* 172 (1995) 1013-1022.
- Dean, D., Schachter, J., Dawson, C. and Stephens, R.: Comparison of the major outer membrane protein variant sequence regions of B/Ba isolates: a molecular epidemiologic approach to *Chlamydia trachomatis* infections. *J Infect Dis* 166 (1992) 383-392.
- Dhir, S., Agarwal, L., Detels, R., Wang, S. and Grayston, J.: Field trial of two bivalent trachoma vaccines in children of Punjab Indian villages. *Am J Ophthalmol* 63 (1967) 1639-1649.
- Frost, E., Deslandes, S., D, G., Bourgaux-Ramoisy, D. and Bourgaux, P.: Variation outside variable segments of the major outer membrane protein

- distinguishes trachoma from urogenital isolates of the same serovar of *Chlamydia trachomatis*. Genitourin. Med. 71 (1995) 18-23.
- Ghaem-Maghami, S., Bailey, R., Mabey, D., Hay, P., Mahdi, O., Joof, H., Whittle, H., Ward, M. and Lewis, D.: Characterization of B-cell responses to *Chlamydia trachomatis* antigens in humans with trachoma. Infect. Immun. 1997 (1997) 4958-4964.
- Grayston, J., Wang, S.-P., Yeh, L.-J. and Kuo, C.-C.: Importance of reinfection in the pathogenesis of Trachoma. Rev Infect Dis 7 (1985) 717-725.
- Guerra, P., Buogo, A., Marubini, E., Pharm, D. and Ghione, M.: Analysis of clinical and laboratory data of an experiment with trachoma vaccine in Ethiopia. Am J Ophthalmol 63 (1967) 1631-1638.
- Hayes, L., Bailey, R., Mabey, D., Clarke, I., Pickett, M., Watt, P. and Ward, M.: Genotyping of *Chlamydia trachomatis* from a trachoma-endemic village in the Gambia by nested polymerase chain reaction: identification of strain variants. J Infect Dis 166 (1992) 1173-1177.
- Hayes, L., Conlan, J. and Everson, J.: *Chlamydia trachomatis* major outer membrane protein epitopes expressed as fusions with LamB in an attenuated *aroA* strain of *Salmonella typhimurium*; their application as potential immunogens. J Gen Microbiol 137 (1991) 1557-1564.
- Hayes, L., Pacharatana, S., Bailey, R., Hampton, T., Pickett, M., Mabey, D., Watt, P. and Ward, M.: Extent and kinetics of genetic change in the *ompI* gene

of *Chlamydia trachomatis* in two villages with endemic trachoma. J Infect Dis 172 (1995) 268-272.

Hayes, L., Yearsley, P., Treharne, J., Ballard, R., Fehler, G. and Ward, M.: Evidence for naturally occurring recombination in the gene encoding the major outer membrane protein of lymphogranuloma venereum isolates of *Chlamydia trachomatis*. Infect. Immun. 62 (1994) 5659-5663.

Holland, M., Bailey, R., Conway, D., Culley, F., Miranpuri, G., Byrne, G., Whittle, H. and Mabey, D.: T helper type-1 (Th1)/Th2 profiles of peripheral blood mononuclear cells (PBMC); responses to antigens of *Chlamydia trachomatis* in subjects with severe trachomatous scarring. Clin Exp Immunol 105 (1996) 429-435.

Holland, M., Conway, D., Blanchard, T., Mahdi, O., Bailey, R., Whittle, H. and Mabey, D.: Synthetic peptides based on *Chlamydia trachomatis* antigens identify cytotoxic T lymphocyte responses in subjects from a trachoma-endemic population. Clin Exp Immunol 107 (1997) 44-49.

Katz, J., West, K., Khatry, S., LeClerq, S., Pradhan, E., Thapa, M., Shrestha, S. and Taylor, H.: Prevalence and risk factors for trachoma in Sarlahi district, Nepal. Br J Ophthalmol 80 (1996) 1037-1041.

Katz, J., Zeger, S. and Tielsch, J.: Village and household clustering of xerophthalmia and trachoma. Int J Epidemiol 17 (1988) 865-869.

- Lampe, M., Suchland, R. and Stamm, W.: Nucleotide sequence of the variable domains within the major outer membrane protein gene from serovariants of *Chlamydia trachomatis*. *Infect. Immun.* 61 (1993) 213-219.
- Lampe, M., Wong, K. and Stamm, W.: Sequence conservation in the major outer membrane protein gene among *Chlamydia trachomatis* strains from the upper and lower urogenital tract. *J. Infect. Dis.* 172 (1995) 589-592.
- Mabey, D., Robertson, J. and Ward, M.: Detection of *Chlamydia trachomatis* by enzyme immunoassay in patients with trachoma. *Lancet* ii (1987) 1491-1492.
- Morrison, R., Belland, R., Lyng, K. and Caldwell, H.: Chlamydial disease pathogenesis: the 57-kD chlamydial hypersensitivity antigen is a stress response protein. *J. Exp. Med.* 170 (1989a) 1271-1283.
- Morrison, R., Lyng, K. and Caldwell, H.: Chlamydial disease pathogenesis: ocular hypersensitivity elicited by a genus-specific 57-kD protein. *J Exp Med* 169 (1989b) 663-675.
- Morrison, R., Manning, D. and Caldwell, H.: Immunology of *Chlamydia trachomatis* infections: immunoprotective and immunopathogenetic responses. In: Quinn, T. (Ed.), *Sexually transmitted diseases*. Raven Press, Ltd., New York, 1992, pp. 57-84.
- Munoz, B. and West, S.: Trachoma: the forgotten cause of blindness. *Epidemiologic Reviews* 19 (1997) 205-217.

- Murdin, A., Su, H., Manning, D., Klein, M., Parnell, M. and Caldwell, H.: A poliovirus hybrid expressing a neutralizing epitope from the major outer membrane protein of *Chlamydia trachomatis* is highly immunogenic. *Infect Immun* 61 (1993) 4406-4414.
- Nichols, R., Bell, S.J., Haddad, N. and Bobb, A.: Studies on trachoma: VI. Microbiological observations in a field trial in Saudi Arabia of bivalent trachoma vaccine at three dosage levels. *Am J Trop Med & Hyg* 18 (1969) 723-730.
- Nichols, R., Bell, S.J., Murray, E., Haddad, N. and Bobb, A.: Studies on trachoma: V. Clinical observations in a field trial of bivalent trachoma vaccine at three dosage levels in Saudi Arabia. *Am J Trop Med & Hyg* 15 (1966) 639-647.
- Peeling, R. and Brunham, R : Chlamydiae as pathogens: new species and new issues. *Emerging Infectious Diseases* 2 (1996) 307-319.
- Prost, A. and Negrel, A.: Water, trachoma and conjunctivitis. *Bull World Health Organ* 67 (1989) 9-18.
- Ramsey, K. and Rank, R.: Resolution of chlamydial genital infection with antigen-specific T-lymphocyte lines. *Infect Immun* 59 (1991) 925-931.
- Schachter, J. and Dawson, C.: The epidemiology of trachoma predicts more blindness in the future. *Scand J Infect Dis, Suppl.* 69 (1990) 55-62.
- Schachter, J., Moncada, J., Dawson, C., Sheppard, J., Courtright, P., Said, M., Zaki, S., Hafez, S. and Loinez, A.: Nonculture methods for diagnosing

chlamydial infection in patients with trachoma: a clue to the pathogenesis of the disease? *J Infect Dis* 158 (1988) 1347-1352.

Sowa, S., Sowa, J., Collier, L. and Blyth, W.: Trachoma vaccine field trials in the Gambia. *J. Hyg.* 67 (1969) 699-717.

Starnbach, M., Bevan, M. and Lampe, M.: Protective cytotoxic T lymphocytes are induced during murine infection with *Chlamydia trachomatis*. *J. Immunol.* 153 (1994) 5183-5189.

Stephens, R., Sanchez-Pescador, R., Wagar, E., Inouye, C. and Urdea, M.: Diversity of *Chlamydia trachomatis* major outer membrane protein genes. *J. Bacteriol.* 169 (1987) 3879-3885.

Su, H. and Caldwell, H.: Immunogenicity of a chimeric peptide corresponding to T helper and B cell epitopes of the *Chlamydia trachomatis* major outer membrane protein. *J. Exp. Med.* 175 (1992) 227-235.

Su, H. and Caldwell, H.: Immunogenicity of a synthetic oligopeptide corresponding to antigenically common T-helper and B-cell neutralizing epitopes of the major outer membrane protein of *Chlamydia trachomatis*. *Vaccine* 11 (1993) 1159-1166.

Su, H. and Caldwell, H.: CD4⁺ T cells play a significant role in adoptive immunity to *Chlamydia trachomatis* infection of the mouse genital tract. *Infect. Immun.* 63 (1995) 3302-3308.

- Su, H., Morrison, R., Watkins, N. and Caldwell, H.: Identification and characterization of T helper cell epitopes of the major outer membrane protein of *Chlamydia trachomatis*. J. Exp. Med. 172 (1990a) 203-212.
- Su, H., Watkins, N., Zhang, Y.-X. and Caldwell, H.: *Chlamydia trachomatis* host cell interactions: role of the chlamydial outer membrane protein as an adhesin. Infect. Immun. 58 (1990b) 1017-1025.
- Taylor, H.: Ocular models of chlamydial infection. Rev Infect Dis 7 (1985) 737-739.
- Taylor, H., Rapoza, P., West, S., Johnson, S., Munoz, B., Katala, S. and Mmbago, B.: The epidemiology of infection in trachoma. Investigative Ophthalmology & Visual Science 30 (1989a) 1823-1833.
- Taylor, H., Siler, J., Mkocha, H., Munoz, B. and West, S.: The natural history of endemic trachoma: a longitudinal study. Am. J. Trop. Med. Hyg. 46 (1992) 552-559.
- Taylor, H., Velasco, F. and Sommer, A.: The ecology of trachoma: an epidemiological study in southern Mexico. Bull World Health Organ 63 (1985) 559-567.
- Taylor, H., West, S., Mmbaga, B., Katala, S., Turner, V., Lynch, M., Munoz, B. and Rapoza, P.: Hygiene factors and increased risk of trachoma in central Tanzania. Arch Ophthalmol 107 (1989b) 1821-1825.
- Thylefors, B.: Development of trachoma control programs and the involvement of national resources. Rev. Infect. Dis. 7 (1985) 774-776.

- Thylefors, B., Dawson, C., Jones, B., West, S. and Taylor, H.: A simple system for assessment of trachoma and its complications. Bull WHO 65 (1987) 477-483.
- Thylefors, B., Negrel, A.-D., Pararajasegaram, R. and Dadzie, K.: Global data on blindness. Bull World Health Organ 73 (1995) 115-121.
- Tielsch, J., West, K., Katz, J., Keyvan-Larijani, E., Tizazu, T., Schwab, L., Johnson, G., Chirambo, M. and Taylor, H.: The epidemiology of trachoma in southern Malawi. Am. J. Trop. Med. Hyg. 38 (1988) 393-399.
- Van de Laar, M., Van Duynhoven, Y., Fennema, J., Ossewaarde, J., Van der Brule, A., Van Doornum, G., Coutinho, R. and Van den Hoek, J.: Differences in clinical manifestations of genital chlamydial infection related to serovars. Genitourin Med 72 (1996) 261-265.
- Wang, S.-P. and Grayston, J.: Trachoma vaccine studies in monkeys. Am. J. Ophthalmol. 63(Suppl.) (1967) 1615-1630.
- Wang, S.-P. and Grayston, J.: Three new serovars of *Chlamydia trachomatis*: Da, Ia and L_{2a}. J. Infect Dis 163 (1991) 403-405.
- West, S. and Bobo, L.: Trachoma, Recent Advances in Pediatrics. Churchill Livingston, London, 1995, pp. 177-188.
- West, S., Congdon, N., Katala, S. and Mele, L.: Facial cleanliness and risk of trachoma in families. Arch Ophthalmol 109 (1991a) 855-857.

- West, S., Lynch, M., Turner, V., Munoz, B., Rapoza, P., Mmbaga, B. and Taylor, H.: Water availability and trachoma. *Bull World Health Organ* 67 (1989) 71-75.
- West, S., Munoz, B., Bobo, L., Quinn, T., Mkocho, H., Lynch, M., Mmbaga, B. and Viscidi, R.: Nonocular chlamydial infection and risk of ocular reinfection after mass treatment in a trachoma hyperendemic area. *Invest Ophthalmol Vis Sci* 34 (1993) 3194-3198.
- West, S., Munoz, B., Lynch, M., Kayongoya, A., Chilangwa, Z., Mmbaga, B. and Taylor, H.: Impact of face-washing on trachoma in Kongwa, Tanzania. *Lancet* 345 (1995) 155-58.
- West, S., Munoz, B., Lynch, M., Kayongoya, A., Mmbaga, B. and Taylor, H.: Risk factors for constant, severe trachoma among preschool children in Kongwa, Tanzania. *Am J Epidemiol* 143 (1996) 73-78.
- West, S., Munoz, B., Turner, V., Mmbaga, B. and Taylor, H.: The epidemiology of trachoma in central Tanzania. *Int J Epidemiol* 20 (1991b) 1088-1092.
- West, S., Rapoza, P., Munoz, B., Katala, S. and Taylor, H.: Epidemiology of ocular chlamydial infection in a trachoma-hyperendemic area. *J Infect Dis* 163 (1991c) 752-756.
- West, S. and Taylor, H.: Reliability of photographs for grading trachoma in field studies. *Br J Ophthalmol* 74 (1990) 12-13.
- Whittum-Hudson, J., An, L., Saltzman, W., Prendergast, R. and MacDonald, A.: Oral immunization with an anti-idiotypic antibody to the exoglycolipid

antigen protects against experimental *Chlamydia trachomatis* infection.

Nature Med 2 (1996) 1116-1121.

William, D., Grubbs, B., Schachter, J. and Magee, D.: Gamma interferon levels during *Chlamydia trachomatis* pneumonia in mice. Infect. Immun. 61 (1993) 3556-3558.

Woolridge, R., Grayston, J., Chang, I., Cheng, K., Yang, C. and Cheng, K.: Long-term follow-up of the initial (1959-1960) trachoma vaccine field trial on Taiwan. Am J Ophthalmol 63 (1967) 1650-1653.

Yuan, Y., Zhang, Y., Watkins, N. and Caldwell, H.: Nucleotide and deduced amino acid sequences for the four variable domains of the major outer membrane proteins of the 15 *Chlamydia trachomatis* serovars. Infect. Immun. 57 (1989) 1040-1049.

Zhang, Y.-X., Stewart, S., Joseph, T., Taylor, H. and Caldwell, H.: Protective monoclonal antibodies recognize epitopes located on the major outer membrane protein of *Chlamydia trachomatis*. J Immunol 138 (1987) 575-581.

Vitae

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EDUCATION

1993 Sep	Ph.D. Student	Div. of Infectious Disease Epidemiology Dept. of Epidemiology School of Hygiene & Public Health The Johns Hopkins University Baltimore, Maryland (1993-present)
1993 Jun	M. Sc.	Div. of Epidemiology Institute of Public Health College of Medicine National Taiwan University Taipei, Taiwan (1991-1993)
1991 Jun	B. Public Health	Dep. of Public Health College of Medicine National Taiwan University Taipei, Taiwan (1987-1991)

RESEARCH / EMPLOYMENT EXPERIENCE

(A) Research

3 /95 – present	Graduate Student on “Molecular Epidemiologic Study on Ocular Chlamydial Infection in a Trachoma endemic Area”
3 /97 -- 1 /98	Research Assistant on “Trichiasis Follow-up Study”
12/95 – 11/97	Research Assistant on “ Azithromycin Clinical Trial On Trachoma “

10/94 – 12/94	Research Assistant on “Needle Exchange Program in Baltimore”
9 /92 -- 6 /93	Graduate Student on “Seroepidemiology of Hepatitis C Virus in Lambay Islet, Taiwan”
1 /92 – 8 /92	Research Assistant on “Seroepidemiology of Dengue Virus in Southern Taiwan”
9 /91 -- 6 /93	Graduate Student on “Seroepidemiology of Dengue Virus and HBV in Lambay Islet, Taiwan”
11/90 -- 6 /93	Graduate Student on “Seroepidemiology of HBV and Immunization of 2 nd Generation HB Vaccine at National Taiwan University, Taiwan”

(B) Teaching

9 /96 – 10/96	Teaching Assistant on “ Public Health Aspects of HIV Infection and AIDS” at the Johns Hopkins Montgomery County Center
3 /92 -- 6 /92	Teaching Assistant on “Communicable Disease Control” at National Taiwan University, Taiwan

(C) Volunteer Work

6 /92 -- 12/92	Counselor on “Vaccine Investigation Group & Arthritis Investigation Group of National Taiwan University Health Care Service Team in Tai-Hsi, Taiwan”
7 /91	Member of “Evaluation of Japanese Encephalitis Vaccine in Lambay Islet, Taiwan”
7 /90	Member of “National Taiwan University Health Care Service Team at Green Islet, Taiwan”

ACADEMIC HONORS, AWARDS

5 /97	Student Travel Grant for Attending 97 th General Meeting American Society for Microbiology(ASM), Miami Beach, FL from ASM
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- 2 /97 Graduate Student Travel Grant for Attending Third Meeting of The European Society for Chlamydia Research, Vienna, Austria from Taipei Economic and Cultural Representative in the United States
- 10/95 Financial Support from the Doctoral Thesis Research Fund from the Summer Program, Department of Epidemiology, the Johns Hopkins University
- 9 /95 Student Travel Grant for Attending 35th Interscience Conference on Antimicrobial Agents and Chemotherapy(ICAAC), San Francisco, CA from American Society for Microbiology
- 9 /91 – 8 /92 Scholarship from Institute of Public Health, National Taiwan University, Taipei, Taiwan
- 1 /91 Honorably Selected to Enter the Graduate Program of Institute of Public Health, National Taiwan University without General Graduate Entrance Examination
- 9 /89 Book Coupon Award (top 5% of the class) from National Taiwan University

AFFILIATION

- 1995 - present Member of American Society for Microbiology
- 1994 - present Member of The Society for Epidemiologic Research
- 1993 - 1994 Member of The Student Outbreak Response Team, the Johns Hopkins University

COMPUTER EXPERIENCE

- | | |
|-------------------|-------------------------------------|
| Word Processing | - Word Perfect, MS Word |
| Statistical | - SAS |
| Graphics | - Harvard Graphics, MS Powerpoint |
| Database | - dBASE, Paradox, EpiInfo, MS Excel |
| Sequence Software | - PHYLIP, ESEE3S, VESPA |
| Internet | - File Transfer, Internet Phone |

SPECIAL INTERESTS

Tennis

LANGUAGES

Proficient in Mandarin and Taiwanese
Familiarity with Swahili

LABORATORY TECHNIQUES

PCR (including designing primers)
PCR-EIA for *C. trachomatis*
Electrophoresis
DNA Cloning
Tissue Cell Culture
Microscopy
Nucleotide Amplification Diagnostics (Ligase Chain Reaction) for *C. trachomatis*
Serology for HBV (Radio-immunoassay) and HCV (EIA)
Plaques Reduction Neutralization Test (PRNT) for Dengue
Hemagglutination Inhibition (HI) Test for Dengue

PUBLICATIONS

JOURNAL PAPER

1. Bobo L, Novak NG, Vitale S, Mkocha H, **Hsieh Y-H**, Munoz B, Quinn TC, West S. Human studies on the association of cellular immune factors in trachoma with infection and disease. *Infectious Diseases in Obstetrics and Gynecology* 1996; 4:177-179.
2. Bobo L, Novak N, Munoz B, **Hsieh Y-H**, Quinn T, West S. Severe disease in children with trachoma is associated with persistent *Chlamydia trachomatis* infection. *Journal of Infectious Diseases* 1997; 176:1524-30.

MANUSCRIPTS IN PREPARATION

1. **Hsieh Y-H**, West S. K., Quinn T.C., Bobo L. DNA sequencing of *Chlamydia trachomatis* major outer membrane protein-1 gene indicates separate mechanisms of trachoma endemicity.
2. **Hsieh Y-H**, Bobo L., Quinn T. C., West S. K. Predictors of trachoma and infection in a six-year follow up of children aged one and two.
3. **Hsieh Y-H**, King CC, Ku CC, Chein LJ, Huang ML, Lin HT, Chen WJ. Seroepidemiologic evaluation: History of dengue epidemics, water storage and mosquito control in an islet of Taiwan.

ABSTRACT

1. Munoz B, West SK, Mkocha H, Lynch M, **Hsieh YH**, Bobo L. Seven-year incidence of trichiasis in a cohort of women with and without scarring. ISGEO Congress. 1998
2. **Hsieh Y-H**, West S, Munoz B, Quinn T, Bobo L. Association of *Chlamydia trachomatis* omp-1 genotypes with infection and disease in trachoma in a 6-year follow-up. 97th General Meeting of the American Society for Microbiology, Miami Beach, FL. 1997 (Poster)
3. Bobo L, Novak N, **Hsieh Y-H**, West S, Munoz B. Disease severity in children with trachoma is associated with persistent *Chlamydia trachomatis* infection. Association for Research on Vision & Ophthalmology. Ft. Lauderdale, FL. 1997 (Poster)
4. **Hsieh Y-H**, West S, Munoz B, Lynch M, Quinn TC, Bobo L. Ten years survey of *Chlamydia trachomatis* omp-1 diversity from ocular samples of children living in trachoma-endemic villages. Third European Chlamydia Meeting. Vienna, Austria. 1996 (oral presentation)
5. Novak N, Munoz B, **Hsieh Y-H**, Mkocha H, West S, Quinn TC, Bobo L. Longitudinal comparison of *Chlamydia trachomatis* omp-1 genovar with duration of infection in children from a trachoma endemic village. Third European Chlamydia Meeting, Vienna, Austria. 1996 (poster)

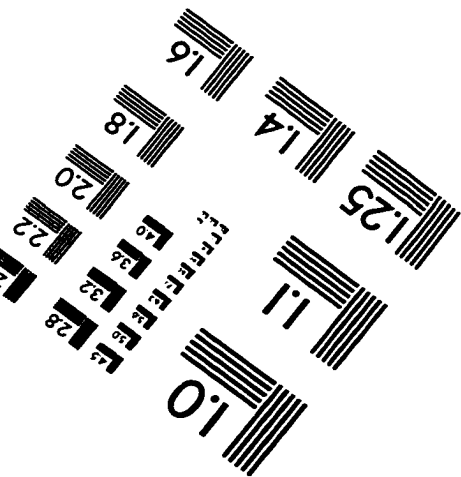
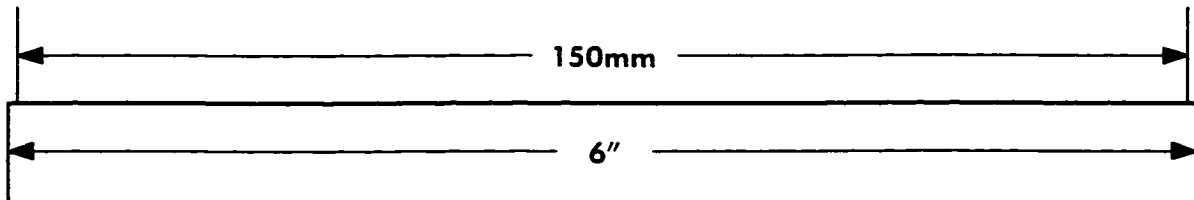
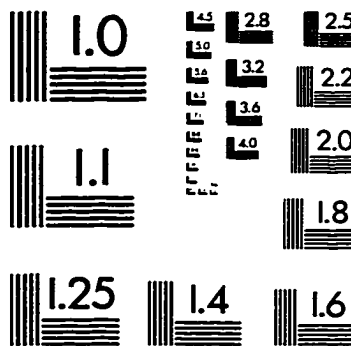
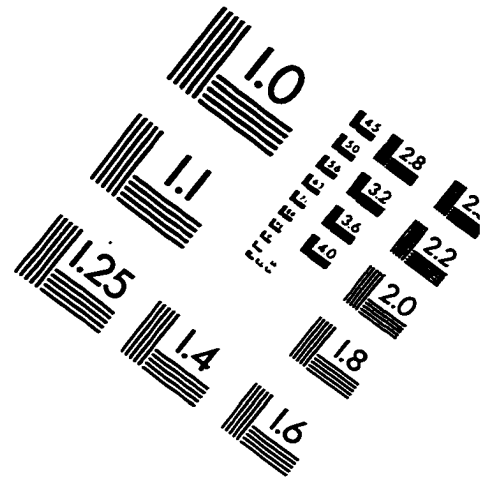
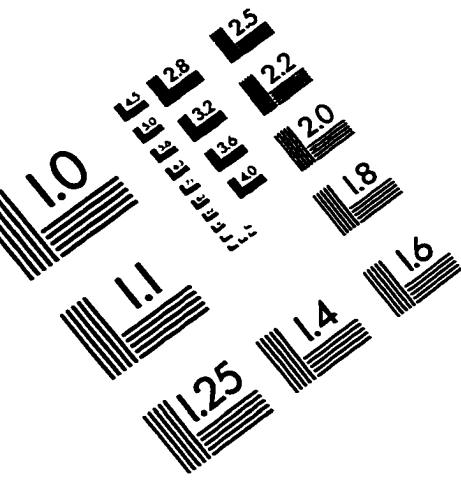
6. **Hsieh Y-H**, West S, Quinn TC, Whittum-Hudson J, Bobo L. Sequencing of *Chlamydia trachomatis* MOMP PCR DNA from direct fluorescent antibody slide samples taken from children in a trachoma area. The 35th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). San Francisco, CA. 1995 (poster)

7. **Hsieh Y-H**, Chiou TY, King CC, Chou HJ, Hseu CW, Kuo PY, Chen DS. Hepatitis B immunization among students at a university in Taiwan. Formosa Medical Association. Taipei, Taiwan. 1991 (oral presentation)
The Third International Symposium on Viral Hepatitis and Hepatocellular Carcinoma. Taipei, Taiwan. 1991 (poster)
The 8th International Congress of Immunology. Budapest, Hungary. 1992 (poster)

8. **Hsieh Y-H**, King CC, Hung SH, Chein LJ, Ku CC, Huang ML, Lin HT, Chen WJ. Seroepidemiology of dengue viral infection in Lambay Islet, Taiwan, 1991.
Formosa Medical Association. Taipei, Taiwan. 1992 (oral presentation)
Sixth Australian Arbovirus Symposium. Brisbane, Australia. 1993 (oral presentation by Dr. CC King)

9. **Hsieh Y-H**, King CC, Ho MS, Lai MY, Horng CB. Assessment of liver function after multiple hepatotropic viral infections. IXth International Congress of Virology. Glasgow, UK. 1993 (poster)

IMAGE EVALUATION TEST TARGET (QA-3)



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