*Epidemiol. Infect.* (1999), **123**, 479–486. Printed in the United Kingdom 

# The duration of human ocular *Chlamydia trachomatis* infection is age dependent

R. BAILEY[1]*, T. DUONG[1], R. CARPENTER[1], H. WHITTLE[2] AND D. MABEY[1]

[1] *London School of Hygiene and Tropical Medicine, Keppel Street, London, UK*
[2] *MRC Laboratories Fajara, PO Box 273, Banjul, The Gambia*

(*Accepted 28 July 1999*)

## SUMMARY

We studied the relationship between age and prevalence, duration and incidence of clinical and laboratory evidence of ocular *Chlamydia trachomatis* infection in a cohort of Gambian subjects examined bi-weekly for 6 months. The duration of disease and infection, estimated by stratified survival analysis, proportional hazards regression and Weibull modelling, was markedly age-dependent. The estimated median duration of disease was 13·2 weeks in 0–4-year-old subjects and 1·7 weeks in those age 15 and over. Adjustment for multiple infections, and for missing observations did not alter this trend. The cumulative incidence rate of disease was reduced threefold with age. More rapid disease resolution is the main source of reduction in prevalence of active trachoma and ocular *C. trachomatis* infection with age; disease incidence was reduced to a lesser extent. This age-dependent resolution may be effected by adaptive cellular immune mechanisms. Mechanisms responsible for natural immunity should receive appropriate emphasis in vaccine design.

## INTRODUCTION

The prevalence of both ocular and genital infection with *Chlamydia trachomatis* has been found to be age dependent. In trachoma endemic areas, clinically active (inflammatory) trachoma, and laboratory evidence of ocular infection with *C. trachomatis*, are very much more prevalent in children than in adults [1, 2]; and a similar phenomenon has been frequently noted in studies of genital *C. trachomatis* infection, where the prevalence has been found to be higher in younger than in older women. This has been documented both in highly exposed groups, such as sex workers and STD clinic attenders and in groups more representative of the general population, as in population based screening studies and studies in antenatal clinic settings [3–6]. It has been found in both developed and developing countries [7, 8].

* Author for correspondence: Department of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, Keppel St, London WC1E 7HT.

The traditional explanation for these observations is that natural infection with *C. trachomatis* results in the development of immunity. It has been assumed by many that such immune responses reduce the incidence of reinfection. Since the early experiments of Murray and colleagues suggested that local (ocular) IgA antibody might prevent ocular reinfection with the guinea-pig inclusion conjunctivitis strain of *Chlaymdia psittaci*, considerable effort has been expended in the search for a vaccine which would stimulate the production of anti-chlamydia IgA at mucosal surfaces [9, 10]. However, more rapid clearance of infection by immune mechanisms in older subjects might also lead to an age-specific reduction in the duration of episodes of clinical disease, and thereby a reduction in disease prevalence. Studies in a variety of animal models, including gene knock-out mice suggest that the immunological mechanisms responsible for the clearance of this intracellular infection differ from those which prevent reinfection, in that they depend on cell mediated rather than

humoral responses [11–13]. Our own studies in trachoma-endemic communities support the hypothesis that cell mediated immune responses to chlamydial antigens play an important role in the clearance of ocular infection and in the resolution of disease [14, 15].

To place the quest for a chlamydial vaccine on a rational footing, and to inform the design of future immune intervention studies, we examined whether the relationship between the prevalence of ocular chlamydial infection and age is due to a reduced duration or a reduced incidence of infection in older age groups. To distinguish between these two possibilities, we studied the relationship between age and the duration of clinical signs of active trachoma, and of ocular chlamydial infection, in all the occupants of 20 households in 2 trachoma endemic villages in The Gambia with 2-weekly follow-up examinations over a 6-month period.

## METHODS

### Subjects

Subjects were recruited from two Gambian villages, Jali and Berending. The environment of both villages has been described elsewhere [16]. Following a baseline survey, eligible households were identified which contained at least one case of active trachoma, and at least four subjects who did not have active trachoma. The study cohort consisted of the members of 20 households, which were selected at random from all eligible households. During the course of the study, all returning household members and in-migrants in the selected households were also included in the study. Subjects in the study cohort were examined at 2-weekly intervals for a total of 13 follow-up visits.

### Clinical examination

The everted upper eyelids of each subject were examined and scored for signs of active trachoma and sequelae, using a $\times 2$ binocular loupe (Dixey Ltd, UK) by an experienced observer (RB) according to the system of Dawson and colleagues [17]. Active trachoma was further graded mild, moderate or severe according to the criteria proposed in this system; mild and moderate disease correspond to follicular trachoma (TF) and severe disease to intense trachoma (TI) in the simplified WHO scoring system [18]. To estimate the error rate in the classification of active disease, a group of subjects from the two village primary schools was examined twice by RB on the same day, and the classifications cross-tabulated. Of 123 subjects examined twice on the same day, 42 subjects were graded as diseased and 81 as disease free at each examination; 2 subjects were classified differently at the first and second examinations, suggesting an observer error rate of about 2%.

Almost all subjects remained asymptomatic and did not receive antimicrobial treatment during the study. Subjects who were symptomatic at the time of examination received a single application of chloramphenicol eye ointment. This occurred rarely. At the conclusion of the study, all subjects who had been documented as diseased or infected received effective treatment for active trachoma which was closely monitored and repeated as necessary.

### Laboratory evidence of infection

To establish laboratory evidence of infection, a cotton-tipped polystyrene swab (Technical Services Consultants) was rubbed three or four times backwards and forwards along the tarsal conjunctival surface of the everted eyelid. The swabs were placed in transport medium and processed by the IDEIA ELISA (Dako Ltd, Ely, UK) for the detection of chlamydial lipopolysaccharide antigen according to the manufacturers instructions. The performance of this test in this environment has been documented elsewhere [16, 19].

### Statistical methods

#### *Assumptions*

The data derived from such a study may be represented by sequences representing the outcome of clinical and laboratory observations. At each time point, data are considered positive (1), negative (0), or missing (.). An episode of clinical disease is taken to be an uninterrupted run of positive observations intervening between two negative observations, such as 000111111110000. An episode of microbiological infection may be similarly defined. If positive observations occur at the beginning or end of such a sequence, such as 11100000111111 this sequence contributes data about the minimum duration of an episode; in statistical terms; the data are right censored. If missing data intervene during an episode, this can lead to right censored data, as in 0000.11-

https://doi.org/10.1017/S0950268899003076 Published online by Cambridge University Press

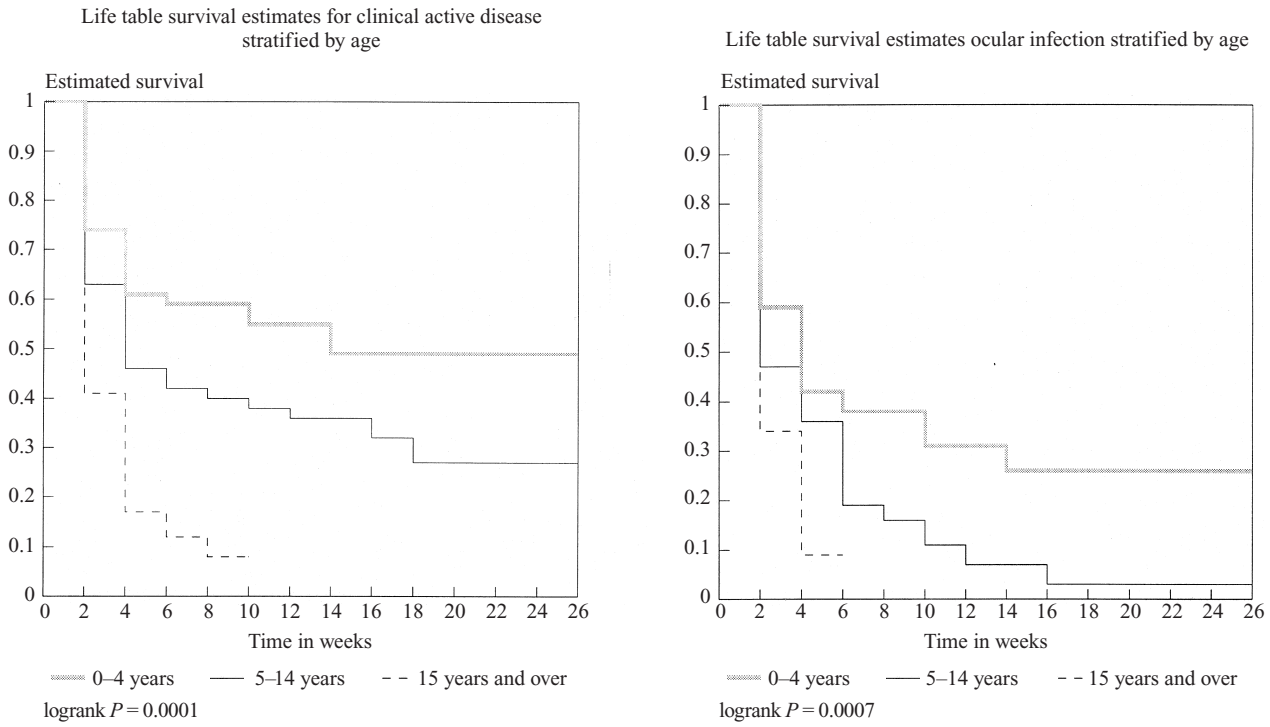


**Fig. 1.** Estimated recovery times for clinically active disease (left panel) and for ocular *C. trachomatis* infection (right panel), stratified by age group.

11100000, but also to sequences which cannot be interpreted such as 00011..11.100 when it is unclear if there are one, two or three distinct episodes, and in the initial analyses, episodes including missing values are excluded. The interval between observations is 2 weeks; for the purposes of analysis, it is assumed that disease and microbiological infection do not resolve and recur within a period shorter than 2 weeks, and moreover that when a change from negative to positive occurs at successive observations, this is assumed to happen at the midpoint between them.

### *Analysis and modelling*

The duration of episodes of disease and infection were analysed using a life-table survival analysis, which was carried out using the SAS procedure LIFETEST (SAS, Cary, NC, USA), in which differences in disease duration, with respect to village sex and age group were analysed. The LIFETEST procedure, which calculates the probability of a disease or infection episode persisting up to each time point and tests the significance of differences using the logrank test (as in Fig. 1), was chosen for its ability to handle right censored data. The joint influences of village, sex and age group were examined using the SAS procedure LIFEREG. This performs proportional hazards regression survival analysis, allowing the joint effects of these covariates on disease or infection duration to be estimated. For convenience and simplicity, the data were examined in three age groups 0–4 years, 5–14 years and 15 years and over: in the proportional hazards regression analysis age was incorporated as a covariate in the model.

To derive estimates of median durations of infection and clinical disease, and to test the assumptions of proportional hazards at all time values and for all age groups, a series of Weibull models were fitted to the survival time data. The possible contribution of missing values was explored by allocating them to be all positive or all negative, and reanalysing the data in these two extreme situations. Finally an attempt was made to allow for the possibility that an apparently single episode of clinical disease might conceal several episodes of infection. Thus, each episode of clinical disease was examined in association with evidence of laboratory infection. If multiple episodes of infection occurred during one episode of disease, each disease episode was regarded as ending at the point where a new episode of infection occurred, and the effect of this assumption was explored in a re-analysis of the data. This is a rather conservative assumption; effectively it allocates the minimum possible duration time to the episode.

https://doi.org/10.1017/S0950268899003076 Published online by Cambridge University Press

## RESULTS

A total of 206 subjects were examined at baseline in the 20 households; 256 subjects were examined altogether in the study. The age/sex distribution and baseline prevalence of active disease in the study cohort are illustrated in Table 1. Fifty-nine of 206 (29%) subjects had active disease at the baseline survey. Seventy-seven (31%) of the 256 of subjects were seen on every possible follow-up occasion; a further 118 (46%) had 1–4 missing observations. Missing observations tended to be negatively associated with disease; where missing observations occurred they were less likely both to have been preceded by, or followed by, an observation of disease, than were non-missing observations (RR = 0·70; 95% CI 0·54–0·92; $P = 0{\cdot}02$ for disease at the preceding observation; RR = 0·78; 95% CI 0·64–0·98; $P = 0{\cdot}02$ for disease at the following observation). When episodes containing missing observations were excluded, there were 131 analysable episodes of clinical disease and 163 analysable episodes of laboratory infection.

The proportion of observations of disease that were intense (TI) in each age group and the proportion of episodes where more than half the disease observations in the episode were of severe disease ('episodes of intense disease') is shown in Table 1. There is a statistically significant trend towards intense disease observations and episodes with increasing age (see footnote to Table 1). With increasing age (over 35) all the disease episodes were intense. The cumulative disease incidence rate (the proportion of subjects negative at entry into the study who subsequently experienced a disease episode) is shown in Table 1. The rate ratio for cumulative incidence in adults compared to young children is 0·38; adults 15 years and over are less likely than children to develop new disease episodes.

### Duration of disease and infection

Lifetable estimates of duration, in the form of survival time curves for episodes of active disease and for infection, stratified by age group, are shown in Figure 1. The effect of age is highly significant, with decreased recovery times for both infection and disease with advancing age. Similar analyses stratified by sex and by village revealed no significant differences in recovery time.

Multivariate analysis using proportional hazards regression also suggested that age was the principal determinant of recovery time, effects of sex and village were not significant. There were too few intense disease episodes for disease severity to be incorporated into the analysis; however there was a tendency for intense disease episodes to be associated with longer durations in the 0–4 age group, and with shorter episodes in those aged 15 and over, but formal tests for interaction were not significant.

Table 2 shows the results of modelling and the effect of various assumptions on the estimated median recovery time for disease. As the distributions are skewed, with some subjects who appeared continuously diseased in the 'tail' the mean durations are likely to be greater than the median durations, and the median is the preferred measure. Table 2 also shows similar data for median recovery time from infection. The probability of recovery was found to vary with the elapsed time the subject had been in the disease state; in young children, the probability of recovery appeared to decrease the longer a subject had been diseased. In contrast, for subjects 15 and over, the longer a person had been diseased or infected the more likely they were to recover. In these and subsequent analyses, therefore, the age groups were modelled separately.

### Adjustment for missing values

If all missing values were taken to be positive, this resulted in a further 29 episodes of disease and a further 32 episodes of infection, i.e. a total of 160 episodes of disease and 195 of infection, whereas if they were all regarded as negative this resulted in a further 55 episodes of disease and 37 episodes of infection; to a total of 186 episodes of disease and 200 of infection. Consideration of these two extreme situations, as shown in Table 2 did not greatly alter median duration except for disease in the 0–4 year group, where exclusion of episodes with missing values may have led to an underestimate of median duration. However in all these analyses a significant effect of age on duration is still apparent, with $P < 0{\cdot}0001$.

### Adjustment for multiple episodes

The results of adjusting the median duration estimates for possible consecutive reinfection are shown in

https://doi.org/10.1017/S0950268899003076 Published online by Cambridge University Press

Table 1. *Baseline data analysed by age group*

| | Age group | | |
|---|---|---|---|
| | 0–4 years | 5–14 years | 15 years or over |
| Number of subjects at baseline | 47 | 86 | 73 |
| Male : female | 24 : 23 | 44 : 42 | 19 : 54 |
| Active disease at baseline (%) | 27 (57) | 29 (34) | 3 (4) |
| Average number of missing observations per person | 2·3 | 2·9 | 3·1 |
| Proportion of disease observations that were intense (TI)* | 27/326 (8%) | 37/355 (10%) | 24/61 (39%) |
| Number of analysed disease episodes | 51 | 54 | 26 |
| Number and proportion (%) of episodes of disease which were intense (TI)† | 4 (8) | 6 (11) | 11 (44) |
| Number of analysed infection episodes | 71 | 77 | 25 |
| Cumulative incidence rate for disease (%) | 13/20 (65) | 25/57 (44) | 15/60 (25) |
| Rate ratio (95% CI) and $P$ value relative to 0–4 year group | 1 | 0·67 (0·44–1·04) $P = 0·17$ | 0·38 (0·22–0·66) $P = 0·003$ |

* $\chi^2$ for trend = 27·3, $P < 0·0001$.
† An episode of disease was regarded as severe if more than 50% of the observations during the episode were of severe disease/TI. $\chi^2$ for trend = 12·4, $P = 0·0004$.

Table 2. *Median disease duration estimates by age group derived from Weibull models*
Median duration (95% CI) estimates for (a) disease, (b) infection episodes in week (median duration (excluding episodes containing missing values))

| Age group | | Missing values all positive | Missing values all negative | Multiple infection episodes |
|---|---|---|---|---|
| (a) Disease | | | | |
| 0–4 years | 13·2 (6·8–28·3) | 11·8 (7·2–20) | 6·8 (4·2–10·8) | 7·2 (4·6–10·7) |
| 5–14 years | 5·3 (3·0–9·2) | 6·6 (4·5–9·5) | 4·2 (2·9–6·1) | 3·9 (2·5–5·8) |
| 15 years and over | 1·7 (1·0–2·9) | 2·1 (1·5–3·8) | 2·1 (1·2–2·5) | 1·6 (1·1–2·1) |
| (b) Infection | | | | |
| 0–4 years | 3·8 (2·4–6·0) | 3·8 (7·2–20) | 3·3 (4·2–10·8) | |
| 5–14 years | 2·1 (1·5–3·2) | 2·8 (4·5–9·5) | 2·0 (2·9–6·1) | |
| 15 years and over | 1·4 (0·9–2·1) | 2·2 (1·5–3·8) | 1·5 (1·2–2·5) | |

Table 2. The effect of the adjustment is to reduce the estimated median duration by approximately 6 weeks in the 0–4 age group, by about 1·5 weeks in the 5–14 age group and there is no effect of adjustment in the 15+year group. However, in the adjusted analysis the effect of age on duration remains statistically significant with $P < 0·0001$.

## DISCUSSION

We have shown that the incidence, clinical features and duration of episodes of active trachoma are significantly modified by age. We have found a similar age-dependence in the duration of episodes of microbiological infection.

https://doi.org/10.1017/S0950268899003076 Published online by Cambridge University Press

Longitudinal studies with frequent follow-up have scarcely been reported in the literature. Taylor and colleagues followed 100 subjects, 41 of whom had disease at baseline, every 3 months for 1 year [20]. In most subjects clinical signs were stable: 13 recovered, 7 acquired new disease and 3 fluctuated. The baseline prevalence of trachoma among the subjects in that study was slightly higher than in the present study (41% *vs* 29%), and thus the children were probably reinfected more often, reducing the chance of observing discrete disease episodes. Other studies have used even longer follow-up periods [21] and were probably even more insensitive to the detection of new disease episodes.

There are some difficulties in generalizing the estimates in this study: the longitudinal sample was chosen deliberately from households which contained both active and inactive cases. The chance of observing new disease episodes is relatively high in these households, because the number of case/non-case pairs is higher than elsewhere. Repeated clinical observations were subject to a 2% error rate. A random 2% binomial error would tend to shorten disease duration as the most common effect is to introduce a single negative into a run of positives, or a single positive into a run of negatives. The error operates to increase the estimated incidence rate, by around 10% for 13 sequential observations. The estimated incidence rate for new disease of about 38% in 6 months in these households is thus likely to be an overestimate of village-wide disease. DNA amplification methods are more sensitive than the IDEIA test we used here to monitor evidence of infection. These methods, which were not established in The Gambia at the time, might have led to a closer correspondence between the estimates of the duration of infection corresponding more closely to the disease duration, and may have reduced the observed number of multiple infection episodes. Missing observations are inevitable in longitudinal studies of this kind; in The Gambia population movements corresponding to times of agricultural effort, schooling and trading contributed to the availability of a subject for study. To assess the potential impact of missing observations, we investigated 'extreme scenarios' where the missing observations were assumed to have been all positive or all negative, but this did not alter the marked age dependence of disease and infection duration.

Active trachoma has been considered to be rare in adults. However, this study suggests that incidence/prevalence effects are partly responsible. The duration of disease is strongly influenced by age; in subjects over 15 median duration is estimated to be 1·7 weeks, about 8 times less than the 13·2 weeks in children under 5. However, the ratio of incidence estimates between adults and children under 5 is considerably narrower, only about 2·5 times less. As most adult episodes resolve within a single follow-up period it is possible that more frequent follow-up (e.g. weekly) might narrow the difference in incidence rates further, or even abolish it altogether. It follows that studies based on prevalence data or infrequent follow-up may underestimate disease episodes in adults relative to children. This needs to be taken into account in the design of future studies of immune intervention where incidence or attack rate for *C. trachomatis* are endpoints; such studies should probably be stratified by age.

It has been difficult to reconcile evidence from animal and migrant studies [25, 26] suggesting that reinfection plays an important role in the pathogenesis of scarring trachoma, with observations that ocular *C. trachomatis* infection of adults is an unusual finding in cross-sectional surveys in regions of endemic trachoma. However in this study 25% of adults experienced one or more episode of ocular disease with an initial point prevalence of 4%. Thus, with pronounced age-dependent shortening of disease and infection episodes, frequent reinfection of adults is compatible with low prevalence rates.

There are obvious difficulties in estimating the duration of genital *C. trachomatis* infection, and only one study has attempted to do so. McCormack and colleagues, reexamined seven women with untreated cervical infection some 15 months after they had first been found to be infected [22]. Four were found to be infected on the second occasion. However it is possible that these women suffered repeated episodes of infection over this period, as their partners had presumably not been treated. The results of this small study were used to estimate that the average duration of untreated genital chlamydial infection in women is 1·5 years, and the duration was assumed for modelling purposes to be similar in men, although no data are available [23]. The World Health Organisation has used the same estimates to calculate the incidence of genital chlamydial infection from the available prevalence data, using the formula

$$\text{incidence} = \frac{\text{prevalence}}{\text{duration}}.$$

Our results suggest that the median duration of ocular

https://doi.org/10.1017/S0950268899003076 Published online by Cambridge University Press

infection with *C. trachomatis* in humans in trachoma endemic areas is considerably less than 1 year. If this can be extrapolated to genital infection, the WHO estimates for the incidence of genital chlamydial infection may be much too low.

Our observation that the duration of chlamydial infection is reduced in older age groups, presumably as a result of acquired immunity, might explain why the prevalence of genital infection in women is often found to decrease with increasing age. This idea is further supported by the results of a recent study by Hook and colleagues who observed that 24 (32%) of 74 subjects in an Alabama STD clinic with culture positive genital infection had spontaneous resolution within 45 days in the apparent absence of effective antichlamydial therapy. They noted that older subjects were significantly more likely to resolve their infections [24].

It is likely that the age-related shortening of disease and infection duration has an immunological explanation, and is the consequence of immune responses stimulated by previous exposure. This concept is supported by the observation that the clinical signs of disease also appear to be modulated by previous exposure, with active disease in adults increasingly associated with gross inflammatory changes, rather than the follicles found in childhood active disease. Adults experience short bursts of intense disease and children prolonged episodes of follicular disease. The immunological mechanisms underlying protection from reinfection with chlamydia have been suggested to involve mucosal sIgA responses and appear to be different from those mediating resolution of infection, in which a number of studies have established a central role for cell-mediated immune (CMI) responses [14]. The significant age-related differences we have observed in the resolution of infection and disease and in the inflammatory response are consistent with the development of effective CMI responses. That these differences underlie much of the apparent reduction in disease prevalence in adult life lends further weight to Morrison's suggestion that the value of a vaccine strategy targeted solely at neutralizing antibody may be debatable and that attempts should be made both to characterize the effectors of CMI responses and to develop vaccine strategies focused on protective CMI, in addition to neutralizing antibody [14].

Differences in disease incidence between adults and children are much less marked than differences in prevalence. The attack rate in adults, though less than that for children, is still substantial. Although some alternative explanations, such as variation in the infectious inoculum required to establish disease, in the number of infectious exposures, and in behavioural determinants of transmission, are inaccessible in this study, we suggest that lasting protective 'sterilizing' immunity rarely develops following natural exposure. Rather than prevented, disease in older patients is instead modified to be more intense and of shorter duration. Whether such modulation of disease is beneficial, by leading to more rapid resolution of infection; or harmful, by contributing to scarring sequelae through the elaboration of pro-inflammatory or fibrogenic cytokines, needs to be investigated. These observations need to be confirmed by longitudinal studies in other environments.

## ACKNOWLEDGEMENTS

The results of this study were presented in part at the Third Meeting of the European Society for Chlamydial Research, Vienna, September 1996. The study was supported by a grant from the Edna McConnell Clark Foundation (to DCWM), and the Medical Research Council (Clinician Scientist Fellowship to RLB).

## REFERENCES

1. Thylefors B, Negrel AD, Pararajasegaram R, Dadzie KY. Global data on blindness. Bull WHO 1995; **73**: 115–21.
2. West SK, Munoz B, Turner VM, Mmbaga BB, Taylor HR. The epidemiology of trachoma in central Tanzania. Int J Epidemiol 1991; **20**: 1088–92.
3. Ramstedt K, Forssman L, Giesecke J, Granath F. Risk factors for *Chlamydia trachomatis* infection in 6810 young women attending family planning clinics. Int J STD AIDS 1992; **3**: 117–22.
4. Hook EW III, Reichart CA, Upchurch DM, et al. Comparative behavioral epidemiology of gonococcal and chlamydial infections among patients attending a Baltimore, Maryland, sexually transmitted disease clinic. Am J Epidemiol 1992; **136**: 662–72.
5. Arno JN, Katz BP, McBride R, et al. Age and clinical immunity to infections with *Chlamydia trachomatis*. Sex Transm Dis 1994; **21**: 47–52.
6. Orr P, Sherman E, Blanchard J, et al. Epidemiology of infection due to *Chlamydia trachomatis* in Manitoba, Canada. Clin Infect Dis 1994; **19**: 876–83.
7. Vuylsteke B, Laga M, Alary M, et al. Clinical algorithms for the screening of women for gonococcal and chlamydial infection: evaluation of pregnant women and prostitutes in Zaire. Clin Infect Dis 1993; **17**: 82–8.
8. Quinn TC, Boateng H, Gaydos C, et al. Community-

based screening for chlamydia and gonorrhoea by urine LCR in rural Uganda and the impact of mass antibiotic treatment. In: *Chlamydia infections*. Stephens RS, et al. eds. Proceedings of the Ninth International Symposium on Human Chlamydial Infections, 1998; 285–8.

9. Murray ES, Charbonnet LT, MacDonald AB. Immunity to chlamydial infections of the eye. 1. The role of circulatory and secretory antibodies in resistance to reinfection with guinea pig inclusion conjunctivitis. J Immunol 1973; **110**: 1518–25.
10. Ward ME. The immunobiological and immunopathology of chlamydial infections. APMIS 1995; **103**: 769–96.
11. Igietseme JU, Ramsey KH, Magee DM, et al. Resolution of murine chlamydial genital infection by the adoptive transfer of a biovar-specific Th1 lymphocyte clone. Reg Immunol 1994; **5**:317–24.
12. Ramsey KH, Rank RG. Resolution of chlamydial genital infection with antigen-specific T-lymphocyte lines. Infect Immun 1991; **59**: 925–31.
13. Morrison RP. Immune protection against *Chlamydia trachomatis* in females. Infect Dis Obstet Gynaecol 1996; **4**: 163–70.
14. Holland MJ, Bailey RL, Hayes LJ, Whittle HC, Mabey DCW. Conjunctival scarring in trachoma is associated with depressed cell-mediated immune responses to chlamydial antigens. J Infect Dis 1993; **168**: 1528–31.
15. Bailey RL, Holland MJ, Whittle HC, Mabey DCW. Subjects recovering from human ocular chlamydial infection have enhanced lymphoproliferative responses to chlamydial antigens compared to persistently diseased controls. Infect Immun 1995; **63**: 389–92.
16. Bailey RL, Hayes LJ, Hampton TJ, Ward ME, Mabey DCW. Polymerase chain reaction for the detection of ocular chlamydial infection in trachoma-endemic communities. J Infect Dis 1994; **170**: 709–12.
17. Dawson CR, Jones BR, Tarizzo ML. A guide to trachoma control. Geneva: WHO, 1981.
18. Thylefors B, Dawson CR, Jones BR, West SK, Taylor HR. A simple system for the assessment of trachoma and its complications. Bull WHO 1987; **65**: 477–83.
19. Mabey DCW, Robertson JN, Ward ME. Detection of *Chlamydia trachomatis* by enzyme immunoassay in patients with trachoma. Lancet 1987; **ii**: 1491–2.
20. Taylor HR, Siler JA, Mkocha HA, Munoz B, West S. The natural history of endemic trachoma, a longitudinal study. Am J Trop Med Hyg 1992; **46**: 552–9.
21. Bailey RL, Kajbaf M, Whittle HC, et al. The influence of local antichlamydial antibody on the acquisition and persistence of human ocular chlamydial infection: IgG antibodies are not protective. Epidemiol Infect 1993; **111**: 315–24.
22. McCormack WM, Alpert S, McComb DE, et al. Fifteen month follow-up study of women infected with *Chlamydia trachomatis*. N Engl J Med 1979; **300**: 123–5.
23. Brunham RC, Cheang M, McMaster J, Garnett G, Anderson R. *Chlamydia trachomatis*, infertility, and population growth in sub-Saharan Africa. Sex Transm Dis 1993; **20**: 168–73.
24. Parks KS, Dixon PB, Richey CM, Hook EW III. Spontaneous clearance of *Chlamydia trachomatis* infection in untreated patients. Sex Transm Dis 1997; **24**: 229–35.
25. Taylor HR, Johnson SL, Prendergast RA, et al. An animal model of trachoma II. The importance of repeated reinfection. Invest Ophthalmol Vis Sci 1982; **23**: 507–15.
26. Detels R, Alexander ER, Dhir SP. Trachoma in Punjabi Indians in British Columbia. A prevalence study with comparisons to India. Am J Epidemiol 1966; **84**: 81–91.