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A longitudinal study of trachoma in a Gambian village: implications concerning the pathogenesis of chlamydial infection

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SUMMARY

In order to investigate risk factors for the acquisition of trachoma, and to study the effect of continued exposure to ocular chlamydial infection on the severity of inflammatory trachoma and its cicatricial sequelae, a longitudinal study was conducted in a Gambian village. Over a 20-month period, the incidence of active (inflammatory) trachoma was significantly higher among those sharing a bedroom with an active case (64/561, 11·4%) than among those who were not exposed in this way (37/658, 5·6%) (relative risk 1·97, 95% confidence interval 1·33–2.90). There was a positive trend in the odds ratio for severe to moderate inflammatory disease versus mild disease as the number of active cases in the bedroom increased, but this failed to achieve statistical significance ($P = 0{\cdot}0506$). Individuals with inflammatory trachoma of moderate or severe intensity at one survey were significantly more likely than others to have moderate or severe inflammatory changes at a previous or subsequent survey (odds ratio 14·9, 95% confidence interval 3·9–68·0), implying that host factors may be more important determinants of severity than the frequency of exposure to reinfection.

INTRODUCTION

Chlamydia trachomatis is among the most prevalent human pathogens. Five to 10% of the sexually active population have been found to have chlamydial genital tract infections in many countries, and some 500 million people are afflicted trachoma, of whom 7 million are blind as a consequence [1, 2].

The inflammatory response to chlamydial infection has the characteristics of a delayed hypersensitivity reaction, and the chlamydial protein which elicits this response has recently been identified as a heat shock protein of 57 kDa [3–5]. It is widely believed, and recent evidence confirms the hypothesis, that subjects with severe inflammatory disease are more likely to develop the fibrotic sequelae which are responsible for most of the morbidity of chlamydial infection [6]. It is also clear that such sequelae are more common after repeated reinfection [7].

Active trachoma can be classified according to the severity of inflammatory changes in the subtarsal conjunctiva [2]. Severe inflammation is more common in

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communities with a high 'pressure of infection', that is, a high prevalence of infection at a young age, and at the community level it has been shown by Nichols and colleagues in Saudi Arabia that more frequent reinfection is associated with more severe inflammatory changes [8].

During the course of a longitudinal study of trachoma in a Gambian village it became clear to us that certain individuals had persistently severe inflammatory disease, as has also been reported by others [9]. The purpose of the present study was to discover whether this could be accounted for by increased exposure of these individuals to reinfection, or whether host factors might be responsible.

Having previously shown that in this village cases of active trachoma are clustered by household and by bedroom [10], we have examined the effect of sharing a bedroom with an active case of trachoma on the incidence of disease over a 20-month period in order to quantify exposure to reinfection.

PATIENTS AND METHODS

Population examined

This study was carried out in the village of Jali, in the West Kiang district of The Gambia. The geography and social anthropology of this region have been previously described [11, 12]. The population of Jali is approximately 950, but as in other rural Gambian villages [13] there is considerable mobility among the population so that the subjects seen at different surveys were not identical.

A register of births and deaths in Jali has been maintained since 1951 by the Medical Research Council Laboratories. At each survey subjects were identified from this register, which had been updated by a census shortly before the first survey in 1984. We were assisted in the identification of subjects by field workers born in the village.

Examination procedure

We examined the entire population of Jali village on three occasions: in November 1984, December 1985 and July 1986. All subjects were examined by a single observer (D.C.W.M.), using a ×4 illuminated monocular loupe. The subtarsal conjunctiva was examined after eversion of the upper eyelid, and the limbus and cornea were also inspected for evidence of pannus or opacities. Findings were scored according to the method of Dawson, Jones and Tarizzo [2] and recorded on a standard proforma.

Inter-observer variability

A total of 162 children selected at random in the village of Jali was examined by both D.C.W.M. and a World Health Organisation team conducting a nationwide trachoma and blindness survey. Agreement as to whether active trachoma was present was reached in 151 subjects (93%). One hundred and forty-three were considered negative by both observers and eight positive. Nine were considered positive by D.C.W.M. and negative by WHO, and two negative by D.C.W.M. and positive by WHO.

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Treatment

During the period of the study one subject, a girl of 10 years with severe inflammatory disease and early entropion, was treated with a 2-week course of oral tetracycline and a 1-month course of tropical tetracycline 1% ointment. All those with trichiasis underwent corrective eyelid surgery in the government hospital in Banjul, by courtesy of Dr H. Faal. At the end of the study all subjects with active trachoma were given a course of treatment supervised by locally employed field workers consisting of a 4-week course of topical 1% tetracycline ointment and a 2-week course of oral tetracycline or, in the case of children under 7 years of age, erythromycin, according to standard recommendations.

Specimen collection and laboratory methods

Swabs were taken from the subtarsal conjunctiva of the upper eyelid for isolation of chlamydia and for the detection of chlamydial antigen by enzyme immunoassay (EIA). In the 1985 survey swabs were collected for both isolation and EIA from a randomized, age-stratified sample of the population. In 1986 swabs for EIA were collected from all subjects examined, but swabs for isolation were collected only from clinically active cases.

Swabs were collected as previously described [14], and stored at −20 °C in the case of EIA specimens and in liquid nitrogen in the case of specimens for isolation. Isolation was carried out in The Gambia using cycloheximide-treated McCoy cells according to the method of Ripa and Mardh [15]. Antigen detection assays were performed in Southampton using the Boots–Celltech IDEIA kit as previously described [14].

Statistical analysis

Statistical analysis was carried out using chi-square and Fisher's exact tests where appropriate. Evidence of confounding was sought by repeated stratifications of potential confounding variables. The influence of the number of active cases per room on severity was examined using the Mantel extension of the chi-square test for trend [16].

Ethical considerations

This study was approved by the joint Gambia Government/Medical Research Council Ethical Committee.

RESULTS

Subjects examined

The numbers of subjects examined at each of the three surveys were 907, 822, and 859. A total of 679 subjects were common to the first two surveys and 661 to the second and third surveys. Five hundred and eighty-six subjects were common to all three surveys. On each occasion it was estimated that at least 95% of those present in the village at the time of the survey were seen. The age-specific prevalence rates for each survey are shown in Fig. 1.

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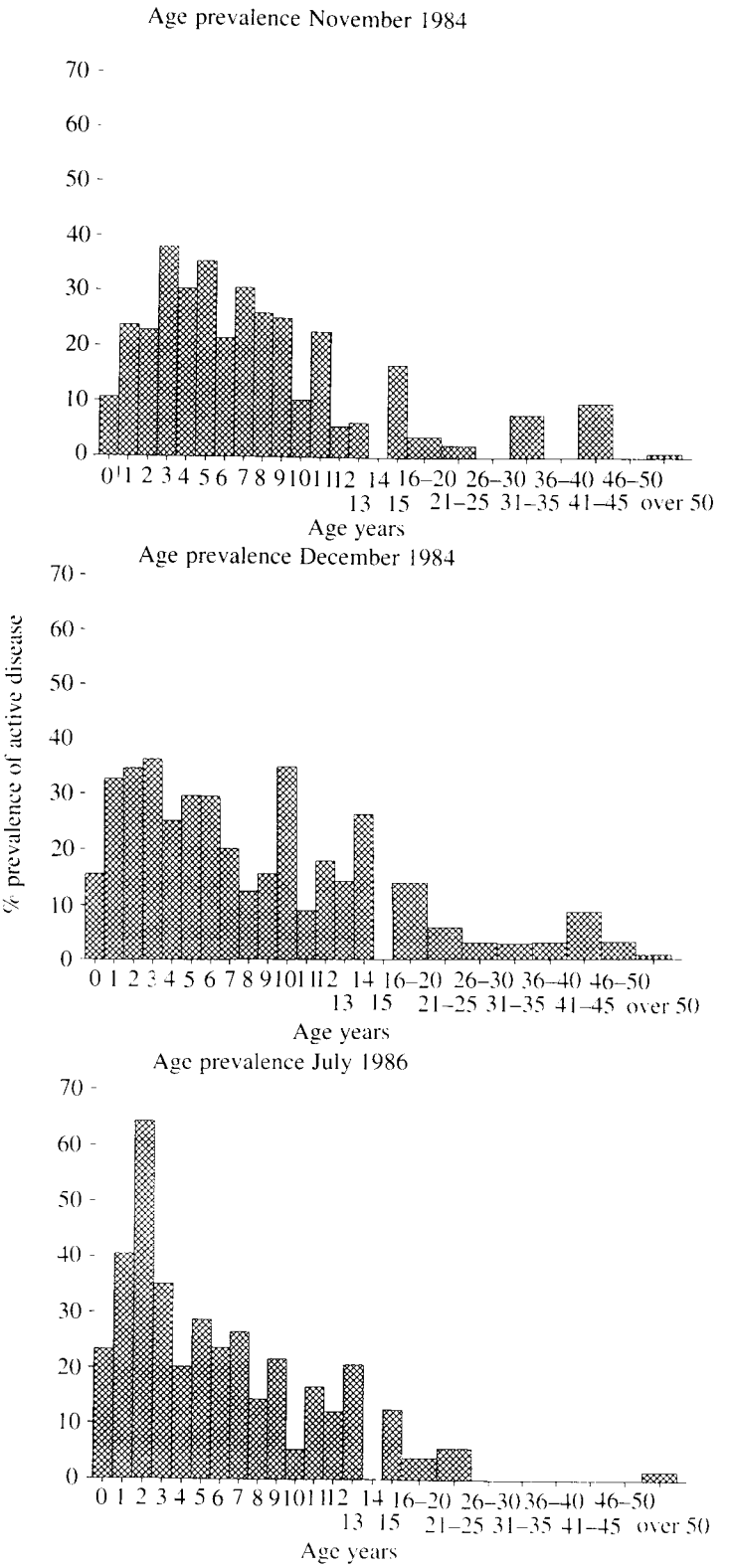

Fig. 1. Age-specific prevalence rates.

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Table 1. *Effect of number of active cases per room on the severity of those cases*

	Number of active cases per room			
Severity	1	2 or 3	4 or 5	6 or 7
Mild	87	95	45	19
Moderate or severe	35	37	28	13
Odds ratio*	1·0	1·14	1·49	2·19

* Odds ratio for proportion of moderate or severe cases compared to that seen in rooms with one active case.

χ^2 for trend (mantel extension) = 3·82; $P = 0·0506$.

Disease activity in relation to microbiological diagnosis

Swabs for the detection of chlamydial antigen were collected from 1225 subjects. A total of 997 of these did not have active trachoma by clinical criteria, 172 had mild, 30 moderate and 26 severe active trachoma. Chlamydial antigen was detected in 5·0, 20·3, 46·7 and 26·9% of subjects in these four categories respectively. The corresponding rates for chlamydial isolation were 3/90 (3·3%), 16/129 (12·4%), 7/21 (33·3%) and 7/19 (36·8%).

Of 49 subjects positive for chlamydial antigen without clinical evidence of inflammatory trachoma, 21 (43%) had conjunctival scarring, compared with 278 of 948 antigen negative subjects (29%). This difference is not statistically significant. However, the prevalence of moderate or severe scarring was significantly higher among antigen-positive than antigen-negative subjects without inflammatory disease (10/49 or 20% vs. 43/948 or 5%; $P < 0·00025$, Fisher's exact test; relative risk 4·50, 95%CI 2·42–11·92).

New cases

During the course of the present study we identified 101 new cases of trachoma, defined as subjects with clinical evidence of active disease who were either not born or were documented as disease-free at a previous survey. Sixty-four of these cases occurred among 561 individuals sharing a room with an active case at a previous survey (11·4%) and 37 among 658 not exposed in this way (5·6%). This difference is significant ($\chi^2 = 12·2$, $P < 0·0005$; relative risk 1·97, 95%CI 1·33–2·90). The relative risk is higher for the comparison between the second and third surveys (42 new cases; relative risk 3·07 95%CI 1·57–5·98) than between the first and second (59 new cases; relative risk 1·51 95%CI 0·92–2·45). There is no evidence of significant confounding by age, sex or number sharing room (data not shown).

Effect of continued exposure on severity of inflammatory disease

The effect of continued exposure on the severity of inflammatory disease can be assessed by comparing severity in bedrooms with different numbers of cases. Table 1 shows the severity scores observed in bedrooms with different numbers of cases. There was no significant difference in the proportion of cases with moderate or severe disease between rooms with one and rooms with more than one active case. Regarding each survey as a separate stratum in the analysis, there is a positive trend in the odds ratio for severe or moderate disease (T2 or T3) versus mild

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Table 2. *Prevalence of moderate/severe scarring among women sharing/not sharing rooms with active cases*

	Sharing with active case	Not sharing with active case
Women aged 30–49 years	14/55 (25%)	18/78 (23%)
Women aged 50 years +	27/53 (51%)	25/72 (35%)

disease (T1) as the number of active cases per room increases but this just fails to achieve statistical significance ($P = 0{\cdot}0506$). In the concatenated data set (all active cases at any survey) there is a significantly higher proportion of severe or moderate cases to mild cases in rooms with four or more active cases compared to those with less than four active cases (41/105 vs. 72/254; $\chi^2 = 3{\cdot}94$, $P = 0{\cdot}047$; odds ratio 1·62 95%CI 0·98–2·68).

Effect of continued exposure on severity of scarring disease

The prevalence of scarring sequelae is higher among women than among men in this community, as has been found elsewhere. In November 1984, 67/237 women aged > 20 years had moderate or severe conjunctival scarring (28%), compared with 26/139 men (19%). This difference is statistically significant ($\chi^2 = 4{\cdot}31$, $P = 0{\cdot}038$). A similar trend is seen in the other two surveys, although it does not attain statistical significance in either of these surveys taken alone. Treating the three surveys as separate strata gives a Mantel–Haenszel weighted odds ratio of 1·41 (95%CI 1·02–1·97) for moderate or severe scarring in females as compared to males in this age group.

It is generally believed that the higher prevalence of sequelae among women is due to their greater exposure to children (the reservoir of infection). In November 1984 over 95% of women shared a room with one or more children compared to less than 10% of men, and these proportions are similar in the other two surveys.

We were unable to show any difference in the prevalence of scarring among women sharing or not sharing a room with an active case over the period of this study (Table 2).

Persistence of severe inflammatory disease

We studied this question by considering all those subjects with active disease in December 1985 who were common to all three surveys, and were classified as mild (T1) or severe (T2 or T3) by the clinical criteria. We then compared the likelihood of these individuals having severe disease at the previous or subsequent survey.

We found that 16/20 (80%) with severe disease in December 1985 also had severe disease at another survey. Of those with mild disease in December 1985, 15/71 (21%) had severe disease at another survey. This difference is highly significant ($P < 10^{-5}$, Fisher's exact test, odds ratio 14·9, 95%CI 3·9–68·0) and indicates that those with severe inflammatory disease tend to have it persistently.

DISCUSSION

There are advantages in studying communities such as Jali in which trachoma is of moderate endemicity. In some environments very high prevalence rates approaching 100% in children make the selection of controls and delineation of risk factors for transmission or severity impossible.

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A number of studies have shown that cases of active trachoma tend to cluster in certain families, and it has been suggested that this might be related to standards of hygiene, in particular the frequency of face washing [17–19]. We have previously demonstrated in Jali that cases of active trachoma are clustered both by compound and bedroom [10, 20]. The reservoir of infection appears to be the eyes of infected children. We have been unable to isolate chlamydia from 90 nasopharyngeal swabs taken from children with trachoma in Jali (unpublished observations) and the prevalence of genital tract chlamydial infection in this village was only 3/98 women of childbearing age [21]. In the present study sharing a bedroom with an active case is a significant risk factor for the acquisition of active disease. This relationship shows no confounding by age, sex or number of persons per room.

Interestingly, sharing a room with an active case is a much stronger risk factor for the comparison of July 1986 with December 1985 (interval 8 months) than for that between December 1985 and November 1984 (interval 13 months). This is likely to be because a shorter interval between surveys renders them more sensitive to transmission events, but some seasonality in transmission cannot be excluded. However Sowa and colleagues [22] found no evidence of seasonal variation in prevalence rates in the Gambian village of Marakissa.

We have used the idea that sharing a bedroom with an active case, as a risk factor for acquisition of active disease, represents an index of exposure to infection. We have studied the effect of this exposure on the severity of inflammatory disease and on scarring sequelae. The proportion of those with severe disease does show an upward trend with increasing numbers of active cases per room; but the effect is a weak one and does not approach statistical significance until there are four or more active cases per room.

Dawson and colleagues observed that in Tunisia severe inflammatory changes tended to persist in certain individuals [9]. They ascribed this to 'a stochastic process determined not only by microbial pathogens but by environmental and host factors'. Taylor and colleagues found a high degree of stability in the clinical signs of trachoma in their longitudinal study of 53 children in Tanzania who were examined every 3 months for a year [23]. The present study, supported by our follow-up of active cases in the intervals between full surveys (unpublished, data not shown) also shows that severe inflammatory changes persist in certain individuals, while others develop only mild changes in response to infection. The weak relationship between exposure to active cases in bedrooms and severity is in contrast to the strong tendency of severe active disease to persist. The possibility of a genetic influence on disease severity and persistence, perhaps mediated through MHC-restricted immune responses, needs to be investigated.

It is generally assumed that those with severe inflammatory disease are more likely to develop scarring sequelae. Dawson and colleagues showed in their analysis of longitudinal data from Tunisia [6] that severe papillary hypertrophy and any degree of conjunctival scarring were predictive of potentially blinding scarring sequelae in the same individual 18 years later. We have not been able to demonstrate any association between the prevalence of scarring disease and exposure to infection over the period of this study.

It is not usually possible to isolate chlamydia from subjects with scarring trachoma and it is not clear why conjunctival scarring should progress in the

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absence of the organism. The possibility of latent infection by non-replicating organisms [24, 25] or of persistent low-grade infection has been under discussion for many years and is given some support by our finding that moderate or severe scarring is more prevalent in antigen-positive than in antigen-negative subjects. However, the migrant studies of Detels and colleagues have shown that scarring does not progress in the absence of continued exposure to reinfection [26].

Probably the progression of scarring disease reflects continuing exposure to infection over a period of years and the period of this study was too short for such an effect to become evident. However, it is surprising that there is not a more pronounced sex difference in the prevalence of scarring sequelae in view of the great disparity in exposure in bedrooms containing active cases between adult males and females. There is no reason to suppose that the pattern of room occupation in Jali has changed in the past few decades.

It seems likely that the risk factors for severity of inflammatory disease and scarring sequelae in this environment differ quantitatively or qualitatively from those determining acquisition of infection. This needs to be investigated by closer study of transmission events and elucidation of further risk factors for transmission, and by investigation of the role of MHC-restricted cell-mediated and humoral immune responses in the pathogenesis of disease.

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