The Epidemiology of Infection in Trachoma

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Specimens for chlamydial isolation culture and direct fluorescent antibody cytology (DFA) were collected from 1671 women and children from a trachoma-endemic area in Central Tanzania. Trachoma was graded using the new World Health Organization grading scheme, and 54% of the children and 9% of the women had inflammatory trachoma (TF or TI). DFA, using the presence of five elementary bodies as the criterion for a positive test, had a sensitivity of 88.0% and a specificity of 87.5% compared to culture and a sensitivity of 54.7% and specificity of 92.8% compared to clinical diagnosis. Altogether, 52.9% of those with trachoma grade TF were positive on either or both culture and DFA versus 77.0% of those with TI. Twenty-nine isolates were serotyped; 18 were serovar A, ten were serovar B, and one was serovar Ba. Positive cultures or DFA were obtained in 6.9% of those graded clinically as not having TF or TI and in a smaller number of those without any perceptible evidence of disease. Conversely, organisms could not be demonstrated in a number of people with severe inflammation (TI) even though some became positive after multiple repeated culture. These two findings of infection without disease and disease without evidence of infection suggest the importance of the immunologic response to infection in determining the clinical status. DFA was found to be an appropriate test for future field studies of trachoma. Further studies of those with disease but without agent and of those with agent but without disease will help understand the dynamics of infection and transmission and the role of the immune response in this important blinding disease.

Repeated episodes of infection with Chlamydia trachomatis are believed to be important in maintaining the sustained inflammatory disease recognized clinically as active or inflammatory trachoma.1,2 Much of this reinfection probably occurs within the family setting, especially between children and women involved in child care.3 The clinical disease in chlamydial eye infection has been characterized as a delayed-type hypersensitivity reaction and can be induced by the topical application of a purified chlamydial antigen.4,5 This immunologic response is seen to lead to conjunctival fibrosis and ultimately to trichiasis and blindness. However, several observations concerning the biology of infection require further elucidation. For example, even in those with the most severe clinical disease, chlamydia frequently cannot be identified in laboratory tests. Only 60% to 70% of those with severe disease and 10% to 20% of those with mild inflammatory disease will be positive on laboratory testing.6-13 Similar observations have been made in a monkey model where, despite the requirement for weekly inoculation with viable organisms to sustain continuing disease, chlamydial cultures are persistently negative after the first few months.2 Even though direct fluorescent antibody cytology (DFA) using monoclonal antibody continued to detect the presence of organisms in repeatedly inoculated monkeys for four weeks longer than culture, it too eventually became negative.14 Similarly, organisms have occasionally been isolated from people who do not have obvious clinical disease.5,11,15

The following study was undertaken to assess the distribution of chlamydial culture and DFA results in families in an area of hyperendemic trachoma to further clarify the association between the presence of identifiable chlamydia and clinical disease. In addition, a newer test for detecting chlamydia, DFA, was compared to chlamydial culture. DFA offers a number of logistic advantages over chlamydial culture in that it does not require the maintenance of a cold chain between collection and testing and is a cheaper, more rapid and less complex test. Lastly, a comparison was made of the frequency with which chlamydia
could be identified within the different grades of trachoma defined by the new simplified grading scheme developed by the World Health Organization (WHO).16

Materials and Methods

Study Population

Specimens were obtained from 1671 women and children who were examined as part of an epidemiologic survey of risk factors for trachoma in Central Tanzania.17 Briefly, a stratified random sample of 20 villages was drawn. Within each village, a cluster sample of preschool children (aged 1 to 7 years inclusive) and their mothers (or female caretakers) were examined. In the first nine villages, members of every second household had specimens collected from their right eye and two photographs taken of the everted upper lid.

Verbal informed consent was obtained from each person or their guardian before entry into this study. The method of obtaining consent and the study procedures had been reviewed and approved by the Joint Committee on Clinical Investigation of the Johns Hopkins University School of Medicine.

Trachoma Grading

One trained examiner used the new simplified WHO trachoma grading scheme throughout the survey.16,18 Each person was examined with a ×2.5 loupe. The presence of five signs were graded for each eye: TF—trachomatous inflammation-follicular; TI—trachomatous inflammation—intense; TS—trachomatous scarring; TT—trachomatous trichiasis; and CO—corneal opacity. Photographs of the tarsal conjunctiva were graded independently in a masked fashion with a ×4 loupe using the same grading scheme.

A sample of 135 photographs was reexamined using a finer grading scheme for the signs TF and TI. The sample included 47 subjects with positive laboratory tests but without the clinical signs of TF or TI and a random sample of 88 other subjects who had either positive laboratory tests or TF or TI. The grading was done without knowledge of either the laboratory results or the previous clinical grade in order to reduce grader bias. For this regrading, each sign was graded as: 0—definitely absent; 1—equivocally present; 2—mild disease definitely present but less than defined by the WHO grade; and 3—equivalent to the WHO grade. This gave a fine grading of TF (FTF) and a fine grading of TI (FTI).

Specimen Collection

 Conjunctival scrapings were obtained from the right superior palpebral conjunctiva of each subject using sterile, dry dacron swabs on plastic shafts. Topical anesthetic was not used. An initial swab on a metal shaft was gently rubbed across the conjunctiva to remove mucus and debris from the tissue surface. A second dacron swab was vigorously stroked across the conjunctiva five times. It was promptly rolled across each half of the central 8 mm well of a fluorescence microscopy slide (MicroTrak Collection Kit, Syva Co., Palo Alto, CA) and then placed into a plastic cryogenic vial containing 1 ml chlamydial transport media composed of 25% fetal calf serum, 10% Eagle's MEM, and 5% DMSO with vancomycin 10 μg/ml, gentamicin 10 μg/ml, and mycostatin 10 μg/ml buffered to pH 7.2.

Chlamydial Culture

Cryogenic vials containing inoculated transport medium were kept at ambient temperature for less than one hour prior to refrigeration at 4°C. One to 8 hr later, vials were snap frozen to −196°C in liquid nitrogen refrigerators. Refrigerators were maintained at this temperature and shipped to Baltimore. Two weeks to 8 months later, specimens were thawed and 100 μl inoculated onto cycloheximide-treated McCoy cell monolayers in each of four wells of a microtiter plate.14 One set of duplicate wells was stained at 2 days (first passage) and another set passed at 2 days and stained at 4 days (second passage) with fluorescein-conjugated monoclonal antibodies to C. trachomatis (MicroTrak Tissue Confirmation Reagents, Syva Co.). Specimens were examined at ×500 magnification and scored positive if one or more typical inclusion bodies were present. The degree of positivity was graded as follows: 1+—one to nine inclusions in the well; 2+—10 to 20 inclusions in the well; 3+—1 to 10 inclusions per ×500 magnification field; 4+—greater than 10 inclusions per ×500 magnification field. The examiner was masked from knowledge of the clinical grading and DFA results. Chlamydial cultures were judged inadequate if the cell monolayers from both the first and second passages were lysed.

There were 69 people who had TI but who were negative on routine tissue culture. Residual collection material was available in 40 of these, and in these instances, the remaining half of the specimen (400 μl) was diluted in culture medium and cultured in three 1-dram vials containing DEAE–Dextran-treated McCoy cells grown on coverslips that were incubated at 37°C in 5% CO2. A one-on-one second passage was...
made after 72 hr, and one coverslip was stained with fluorescein-conjugated monoclonal antibody. If it was negative, the remaining two vials were combined and split to inoculate three fresh vials of cells. A total of six serial passages was finally made before the specimen was considered negative, at which time all three coverslips were examined.

Previously, we had established the comparability of the two culture methods in our laboratory. In an unpublished substudy, replicate swabs were collected from seven monkeys at days 0, 7, 14, 21, 28, 42 and 56 after ocular inoculation with 5000 inclusion forming units (IFUs) of a serovar B organism (TW-5). Microtiter plate and dram vial cultures were performed as described above, except only two passages were used in each culture. The two culture methods gave 86% agreement (42 out of 49 paired cultures). In one case, the vial culture became positive on the second passage while the plate culture remained negative. In six cases, the vial cultures were negative while the plate cultures were positive (once in the first passage and five times in the second passage). In only one of these seven discrepancies did DFA show ten or more elementary bodies (EB). These data suggest that, in our hands, chlamydial culture in a microtiter plate is at least as sensitive if not more sensitive than culture in dram vials. The few discrepancies that occurred did so at lower levels of infection.

DFA Cytology

After collection, slides were air-dried and then flooded repeatedly with acetone for 5 min. Fixed slides were kept at ambient temperature for 1 to 8 hr while collections proceeded. Slides were stored at 4°C for up to 6 weeks prior to shipment to Baltimore. Slides were then stored at -20°C until processing 2 weeks to 8 months later. Prior to staining, slides were allowed to thaw for 30 min, immersed in acetone for 5 min, and air-dried. Thirty microliters of fluorescein-conjugated monoclonal antibody to the major outer membrane protein of C. trachomatis (MicroTrak Direct Reagent, Syva Co.) were placed over the specimen which was then incubated in a moist chamber for 30 min at room temperature, rinsed twice with distilled water, and air-dried. Slides were read at \( \times 500 \) magnification under oil immersion by fluorescence microscopy. Each field of each slide was examined. Fluorescent particles were examined at \( \times 500 \) and confirmed at \( \times 1250 \). Typical apple-green fluorescing EB were enumerated. When less than ten EB were seen in the entire slide, the number of EB was recorded. If ten or more EB were present, the result was graded as follows: 1+—10 to 49 EB per well; 2+—50 to 100 EB per well; 3+—1 to 10 EB per \( \times 500 \) magnification; or 4+—greater than 10 EB per \( \times 500 \) magnification.19 The examiner was masked from knowledge of the clinical grading and culture results. Greater than 200 epithelial cells per slide were required as a criterion of an adequate DFA specimen.

Serotyping

Isolates for serotyping were cultured in 1-dram vials. When 80% infective titers were reached, EB were harvested, washed and resuspended in 0.2 ml of formalin–TWEEN-80–PBS solution (0.02 formaldehyde, 0.01% TWEEN-80, [Sigma Chemical, St. Louis, MO] in sterile PBS pH 7.0). Serotyping was performed using the MicroIF system of Wang and coworkers.20,21 Equal parts (0.1 ml) of the EB suspension and a 3% formalinized yolk sac were combined and pinpoint-sized dots placed on a clean glass slide. These were overlayed with monoclonal antibodies specific for C. trachomatis serovars (Monoclonal Antibody Typing Kit, Washington Research Foundation, Seattle, WA).21

Statistics

Chi-square and Fisher’s exact tests were used to assess differences in proportions. Comparison of DFA and culture tests were done using standard measures of sensitivity, specificity, and predictive values. Unless explicitly stated otherwise, all comparisons and analyses use the routine microtiter plate culture results. Spearman’s Rank correlation coefficient was used to assess the correlation between the laboratory tests and between the laboratory results and the clinical grading. For this analysis, specimens positive on first passage were scored according to their inclusion grading. Specimens that were only positive on second passage were arbitrarily scored as 0.5 as they were obviously positive but presumably of an infectious titer insufficient to be positive on first passage. For DFA results, specimens with 1 to 4 EB were scored as 0.25, 5 to 9 EB as 0.75, and then according to their EB grading.

Results

In all, specimens from 1671 subjects were available for laboratory evaluation for the presence of C. trachomatis (Table 1). Of these specimens, 1090 came from children aged 1 to 7 years (51% were girls; and 54% of all children had inflammatory trachoma), and 581 of the specimens came from adult women who were the children’s mothers or caretakers (9% of women had inflammatory trachoma). Altogether,
Table 1. Age and sex distribution and prevalence of inflammatory trachoma in the 1671 study subjects

<table>
<thead>
<tr>
<th>Age</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percent with TF and/or TI</td>
</tr>
<tr>
<td>&lt;8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-24</td>
<td>133</td>
<td>57%</td>
</tr>
<tr>
<td>25-34</td>
<td>256</td>
<td>10%</td>
</tr>
<tr>
<td>35-44</td>
<td>142</td>
<td>6%</td>
</tr>
<tr>
<td>45+</td>
<td>50</td>
<td>6%</td>
</tr>
<tr>
<td>Total</td>
<td>581</td>
<td>9%</td>
</tr>
</tbody>
</table>

265 specimens were positive on routine chlamydial culture, 237 (89.4%) on the first passage and 28 (10.7%) on the second passage. There were 386 positive specimens on DFA testing, 32 (8.3%) with five to nine EB, and 354 (91.7%) with ten EB or more. In 51 DFA smears, intracytoplasmic inclusions were identified. The number of inclusions ranged from one to 25.

Adequacy of Specimens

Cultures were considered to be inadequate if the cell monolayer had been completely destroyed in both the first and second passage (23 cases) or if it had been completely destroyed in one and had been partially destroyed in the other (12 cases). In most cases, there was frank evidence for bacterial infection. Overall, 35 (2.1%) cultures were inadequate. Inadequate cultures occurred in all age groups without a clear age-specific or sex trend. They were equally common in eyes with TF or TI but were more common in eyes with either TF or TI (combined rates 8.9%) than those without any inflammation (12.7%) ($\chi^2 = 5.94, P = 0.02$). Five times as many inadequate smears corresponded to a negative culture (13.1%) as to a positive culture (2.6%) ($\chi^2 = 24.0, P < 0.001$). This suggests that an inadequate specimen may have been collected and the culture was artifactually negative. However, seven of the 188 inadequate smears (3.8%) were coupled with a positive culture, showing that this did not occur invariably. Only one specimen was judged as being inadequate for both culture and DFA.

Comparison of DFA and Culture

Altogether, both culture and cytology specimens were adequate for 1451 people. The performance of DFA was highly comparable to that of culture (Table 2). As would be expected, the sensitivity, specificity and predictive values changed with the criterion for DFA positivity. The criterion for DFA positive of five or more EB seemed to give the optimal performance as it maximized the balance between sensitivity and specificity. Thus, this criterion has been used in subsequent analyses. There was a strong correlation between the number of inclusions seen on culture and the number of EB seen on DFA (Spearman’s Rank correlation coefficient $0.665, P < 0.0001$) (Table 3).

Correlation of Clinical Disease with DFA and Culture

The frequency of a positive culture or DFA in those with inflammatory trachoma showed no difference by age or sex, although there was a marked increase in the number of positive laboratory results with increasing severity of inflammatory trachoma (Fig. 1). Overall, DFA was positive more frequently than culture (Table 4). If the results of both laboratory tests were combined, 52.9% of those with TF and 77.0% of those with TI were positive, whereas only 3.0%) than in eyes without inflammation (1.5%) ($\chi^2 = 4.45, P = 0.04$). Inadequate cultures were equally common in DFA-positive and DFA-negative (2.3%) cases but less common in DFA-inadequate specimens (0.5%) (Fisher’s exact test, $P = 0.017$).

DFA specimens were considered inadequate if less than 200 cells were seen in the smear. In all, 188 smears (11.3%) were inadequate. Two of these smears were still positive, having more than ten EB, and these have been included in the subsequent analyses. Inadequate smears were more common from the youngest children; 20% of specimens were inadequate in 1- to 2-year-olds compared to 13% in 3- to 7-year-olds and 4% in those over age 7 years ($\chi^2 = 52.3, P < 0.001$). Inadequate smears were less common in eyes with either TF or TI (combined rates 8.9%) than those without any inflammation (12.7%) ($\chi^2 = 5.94, P = 0.02$). Five times as many inadequate smears corresponded to a negative culture (13.1%) as to a positive culture (2.6%) ($\chi^2 = 24.0, P < 0.001$). This suggests that an inadequate specimen may have been collected and the culture was artifactually negative. However, seven of the 188 inadequate smears (3.8%) were coupled with a positive culture, showing that this did not occur invariably. Only one specimen was judged as being inadequate for both culture and DFA.

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Table 3. Correlation between degree of positivity of culture and DFA for 1451 paired specimens

<table>
<thead>
<tr>
<th>DFA grading</th>
<th>Negative* (2nd passage positive)</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>4+</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>988</td>
<td>19</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>1017</td>
</tr>
<tr>
<td>1-4 EB</td>
<td>56</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>58</td>
</tr>
<tr>
<td>5-9 EB</td>
<td>25</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>1+</td>
<td>52</td>
<td>2</td>
<td>28</td>
<td>4</td>
<td>2</td>
<td>88</td>
</tr>
<tr>
<td>2+</td>
<td>19</td>
<td>3</td>
<td>12</td>
<td>2</td>
<td>6</td>
<td>42</td>
</tr>
<tr>
<td>3+</td>
<td>44</td>
<td>10</td>
<td>45</td>
<td>19</td>
<td>3</td>
<td>151</td>
</tr>
<tr>
<td>4+</td>
<td>9</td>
<td>6</td>
<td>14</td>
<td>5</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>1193</td>
<td>124</td>
<td>31</td>
<td>64</td>
<td>11</td>
<td>1451</td>
</tr>
</tbody>
</table>

* Spearman’s Rank correlation coefficient 0.665, \( P < 0.0001 \)

23.0% of those with TS and 22.2% of those with TT in the absence of inflammatory trachoma were positive. The performance of the two laboratory tests were compared with the clinical diagnosis of inflammatory trachoma (either TF or TI) (Table 5). DFA, using five or more EB as the criterion for a positive result, was more sensitive and only slightly less specific than culture. If the criterion for a positive DFA was changed to ten or more EB, the sensitivity declined to 52.5% while the specificity increased to 94.9%. For both culture and DFA, there was a strong correlation between infectious load (as measured by inclusion count and the number of EB) and increasing severity of disease (ranked normal, TF, and TI) (Spearman’s Rank correlation coefficient 0.421, \( P < 0.001 \) for culture; 0.531, \( P < 0.0001 \) for DFA).

Examination of Those not Graded as TF or TI but Laboratory Test-Positive

The photographs of 47 subjects who were graded clinically as not having inflammatory trachoma but who were positive by either chlamydial culture or DFA were regarded using the fine grading scheme as described in Methods. Twenty-seven people were positive by culture; 17 of these were also DFA-positive. The remaining 20 people were positive on DFA alone. Nineteen of the 27 positive cultures (70%) were positive in the first passage. The DFA results were not predictive as to whether the culture would be positive on the first or second passage, although those who were DFA-negative had lower infectious titers on culture. The 17 people who were positive on both culture and DFA had more EB seen on DFA than the 20 people who were positive on DFA but negative on culture. Thus, in each case, the discrepancies between culture and DFA tended to occur with lower levels of organism. There was no overall difference in the age or sex distribution of people who were positive in culture or in DFA nor was there a difference in the occurrence or severity of inflammatory trachoma in other household members. Therefore, for the next analysis, these subgroups were combined and placed in a matrix on the basis of their fine grading score for FTF and FTI (Table 6). Altogether, six people had completely normal appearing conjunctiva but were positive on either or both culture or DFA cytology (Tables 6, 7). A total of 15 had an equivocal status (one or both fine signs graded as 1), and 26 had definite signs of disease that were less than the criteria for being classified as TF or TI (one or both fine signs graded as 2).

These 47 people with positive laboratory tests represent 4.6% of the 1029 people who had neither TF nor TI. Nearly two-thirds of these people were women (Table 7). The rate of positive laboratory tests for those without TF or TI was lowest for girls (2.1%), was intermediate for boys (4.5%), and was highest for women (5.7%) (odds ratio for boys 2.1 [0.77-6.4] and for women 2.7 [1.02-7.0] compared to girls). Those

Table 4. Frequency of positive chlamydial culture or DFA cytology by clinical trachoma status

<table>
<thead>
<tr>
<th>Inflammatory trachoma</th>
<th>Cicatricial trachoma</th>
<th>Number positive</th>
<th>Number total</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture</td>
<td>34/927 (3.7%)</td>
<td>150/481 (31.2%)</td>
<td>73/142 (51.4%)</td>
</tr>
<tr>
<td>DFA</td>
<td>48/813 (5.9%)</td>
<td>221/447 (49.4%)</td>
<td>100/140 (71.4%)</td>
</tr>
<tr>
<td>Culture or DFA</td>
<td>55/800 (6.9%)</td>
<td>229/433 (52.9%)</td>
<td>104/135 (77.0%)</td>
</tr>
</tbody>
</table>

* Without TF or TI.
Table 5. Performance of chlamydial culture and DFA cytology (clinical grading of inflammatory trachoma [TF and/or TI] is the reference standard)

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive</th>
<th>Negative</th>
<th>(Number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>35.8</td>
<td>95.9</td>
<td>84.2</td>
<td>70.8</td>
<td>(1636)</td>
</tr>
<tr>
<td>DFA</td>
<td>54.7</td>
<td>92.8</td>
<td>83.2</td>
<td>75.8</td>
<td>(1485)</td>
</tr>
<tr>
<td>Culture or DFA</td>
<td>58.6</td>
<td>91.6</td>
<td>81.2</td>
<td>77.5</td>
<td>(1451)</td>
</tr>
</tbody>
</table>

with either no inflammation or with equivocal disease had less scarring (TS) than the general population, but the proportion coming from families in which at least one other person had TF was not significantly different from the group as a whole.

Examination of Those with Severe Clinical Disease but Laboratory Test-Negative

There were 69 people who were clinically graded as having TI with or without TF for whom routine cultures were negative. In some wells of negative cultures, debris with an appearance similar to free EB was seen, suggesting that chlamydia may have been present but had not been detected by culture. To see if further blind passage of material might lead to the establishment of inclusions and a positive culture, the remaining collection material was serially cultured in dram vials for the 40 people with TI and negative culture for whom residual material was available. In all, 16 became culture-positive; of 17 DFA positive specimens, seven became culture-positive (41%) and of 23 DFA negative specimens, nine became culture-positive (39%). Although 13 of the 16 became positive by the third passage, three were only positive on the final sixth passage. There was no clear indication of titration of infectivity as nine had six or more inclusions per high-power field on their first positive passage. For children, there was no difference in the age or sex distribution of those who became positive compared to those who remained negative. Four adult women were retested; two became positive (aged 25 and 40 years) and two did not (aged 22 and 70 years). If those who became positive after reculturing in vials were added to those who were positive on routine culture, the overall rate of positive culture in people with TI would become 89 out of 142 (63%). Similarly, the number of positives on first passage would become 84.3%, with 10.0% being detected on second passage and 5.7% on third to sixth passage.

Distribution of Serotypes

Specimens from 69 patients with TF that had been positive on tissue culture were selected for serotyping. They formed two blocks of consecutive specimens collected at the start and the end of the specimen collection. It was possible to raise sufficient material to serotype 29 of these; 18 were serovar A, ten were serovar B, and one was serovar Ba. There was no tendency for the serovar to be grouped by sex or by disease severity. Serovar A tended to be more common in younger children; 15 of the 18 isolates from the 1- to 5-year-olds were serovar A, whereas only three of the 11 isolates from those 6 years or older were serovar A (Fisher's exact test, $P = 0.003$). The serovar Ba came from a 1-year-old girl. Three isolates came from the same family: a 26-year-old woman and her two sons aged 7 and 4 years. Each was serovar B.

Discussion

This study investigated the biology of infection with C. trachomatis in an area of hyperendemic trachoma. It studied a population-based sample of children and their mothers and compared the clinical status with chlamydial culture and DFA. Because it was population-based, the findings relating to sensitivity and predictive value have relevance for other populations in which trachoma is endemic.

Overall, DFA was found to be a very satisfactory test for use in field studies of trachoma. The results obtained with DFA showed a good correlation with
Table 7. Summary of characteristics of 47 people who were positive on laboratory testing but who did not have TF or TI

<table>
<thead>
<tr>
<th>Status</th>
<th>Number</th>
<th>Boys</th>
<th>Girls</th>
<th>Mothers</th>
<th>TS</th>
<th>Culture+, DFA+</th>
<th>Culture+, DFA-</th>
<th>Culture-, DFA+</th>
<th>Family members with TF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (both 0)</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>67%</td>
</tr>
<tr>
<td>Equivocal (at least one fine grade 1)</td>
<td>15</td>
<td>4</td>
<td>0</td>
<td>11</td>
<td>1</td>
<td>(7%)</td>
<td>3</td>
<td>4</td>
<td>80%</td>
</tr>
<tr>
<td>Mild disease (at least one fine grade 2)</td>
<td>26</td>
<td>8</td>
<td>4</td>
<td>14</td>
<td>6</td>
<td>(27%)*</td>
<td>12</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>

* One woman aged 28 had both TS and TT.

Both culture results and with clinical status. DFA offered one advantage over culture as it was obvious when an inadequate DFA specimen had been collected. However, inadequate DFA specimens were recognized in 11% of cases. Inadequate DFA specimens were more common in young children and in those without ocular inflammation. DFA specimens can be masked by mucus; therefore it is important to clear any gross discharge from the eye before obtaining the specimen. Inadequate DFA specimens contained insufficient cells, and this occurs either when the specimen is not collected with enough vigor or when it is not transferred firmly enough from the swab to the slide. That the latter occurs is demonstrated by the finding of more than five EB or the occurrence of positive culture tests in specimens where less than 200 cells were seen in the DFA smear. Careful attention to specimen collection can reduce the rate of inadequate specimens.

With chlamydial culture, it is not possible to tell if an inadequate specimen has been collected. Cultures recognized as being inadequate usually result from bacterial contamination, most commonly from concurrent bacterial infection, and antibiotics are included in the collection and culture media to reduce this. The rate of 2.1% inadequate cultures is acceptable and compares very favorably with other reports, albeit using genital specimens.22 Our culture specimens were frozen in liquid nitrogen, and this probably caused some reduction in infectious titer of chlamydia;23 but, once frozen, the variation in the duration of storage should not have led to a change in titer.8 Cultures were stained with a DFA reagent, which is reported to be more sensitive than iodine stain.14,24,25

Recently, some investigations have reported improved results with the DFA test if methanol is used as a fixative instead of acetone26,27 and this change has subsequently been recommended by the manufacturer. It is thought that methanol removes some surface components that may expose more epitopes for the monoclonal antibody reagent to recognize. This effect is not thought to occur with a brief acetone fixative but may occur with prolonged acetone fixation. We used a prolonged acetone fixation in the field, which was also repeated in the laboratory prior to staining, and our results compare more than favorably to those using methanol fixation.27

There was good agreement between the two laboratory tests. As would be expected, the performance of DFA compared to culture varied with the criterion for a positive test. Initially, a finding of ten EB was recommended for the criterion for a positive DFA test.28 Many have used this cutoff,10,11,13,26-29 although others have suggested five,30-33 three,14,34 two35 or even one EB11,36 as being sufficient for the diagnosis.

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**Fig. 1.** Proportion of positive laboratory tests by clinical trachoma grading.
of a positive smear. Our data would suggest that five EB is the optimal cut-off for ocular specimens, although it should be recognized that bacterial contamination is much more common with genital and rectal specimens and a higher cutoff may still be appropriate for these specimens.

Both laboratory tests correlated well with the clinical grading. This is the first report to compare the microbiologic status with the new simplified WHO trachoma grading scheme, and our findings provide a laboratory validation of this system. An increasing laboratory identification rate with increasing clinical severity of inflammation has been reported by others. Others have also identified a small number of people who were seemingly normal but from whom organisms can be identified—"asymptomatic carriers." Rates of positive tests in such people have varied from about 2% to 4% to 5%. It is possible that in some cases this could represent laboratory error or contamination, which Schachter has reported can occur at a rate of 0.5%. However, we found 47 people who did not have TF or TI and who were positive on laboratory testing, some by culture alone and some by DFA alone, but 17 (36%) by both tests. Over half of these people had mild clinical disease that was not of sufficient severity to meet the criteria to be graded as either TF or TI and another third had equivocal clinical findings. However, of the 47 people, there were six (13%) who had entirely normal-appearing eyes five were women, none of whom had trachomatous scarring. It is interesting to speculate whether these six people were incubating their initial infection, as suggested by Nichols, or whether they may be true asymptomatic carriers whose immunologic status is somehow altered so they can tolerate the presence of chlamydia without producing an immunopathologic response. Clearly, such a group of patients is worthy of further investigation.

Of equal interest was the finding that 33% of patients with severe clinical disease (TI) were initially negative by laboratory testing. This finding is in line with previous reports, although the actual percentage varies somewhat as different clinical grading schemes and laboratory tests were used. Each laboratory test used to detect chlamydia clearly does not have 100% sensitivity, although culture with serial passage does offer the possibility of amplification of infectious particles that would lead to an increased sensitivity that is not possible with other tests. Most specimens found to be positive on culture were positive during the first passage. The first passage has a reported sensitivity which can vary between 65% to 97% compared to the overall culture results. A second blind passage is often used routinely for chlamydial culture. We were interested to see if additional blind passages of specimens from cases with severe inflammatory disease (TI) would lead to a further increase in positive cultures as has been reported by others for genital cultures. Although others have shown the general comparability of chlamydial culture in microtiter plates and vials, we believed it important to first validate the methods we use in our laboratory as outlined above.

With the material from the patients in Tanzania, we found an enhanced sensitivity of culture in vials when serial passage was used. Of the 40 specimens from patients with TI that were initially negative, 19 became positive when passed up to six times in vials. The reason for increased sensitivity with multiple blind passage is unclear. It is possible that the infectious material could be clumped in the collection medium and was not included in the original inoculum. This tendency should be minimized by vortexing prior to specimen transfer. Preformed tear antibodies or other cytokines could prevent organism replication, but given the marked dilutional effect and the fact it only occurs in some specimens, this seems unlikely. Length of storage was not correlated with a delayed culture, although unrecorded minor variations in initial specimen preparation and handling cannot be excluded. It is possible that some biologic variation in the ease with which the organism adapts to cell culture could account for the appearance of delayed positives. Multiple blind passage did increase the number of culture positive specimens; however, we would not recommend this labor-intensive and extensive effort for the routine culture of chlamydia and believe that in most circumstances a second blind passage is adequate.

It is of interest that the DFA results did not predict which specimens became positive on reculture in vials, although the finding that some people with a positive DFA test had negative cultures suggests the presence of nonviable organisms in these smears. However, despite the intensive efforts to identify organisms, there were still 14 people with severe inflammatory disease who did not have demonstrable organism by either culture or DFA and ten who had organism seen on DFA but who were culture-negative. These people form a most interesting subgroup, further study of whom may provide insights into the pathogenesis of the disease.

Parallels for the presence of clinical disease in the absence of demonstrable organisms have been found in the animal models of trachoma. Despite the need for repeated weekly inoculation of viable organisms to sustain chronic disease, chlamydia cannot be isolated or identified after the first few months.
tivization that is characteristic of trachoma. This suggests that organisms that are not actively replicating may be able to elaborate or release sufficient antigenic material to sustain the conjunctival inflammatory response. It is known that penicillin, for example, inhibits chlamydial replication but does not stop production of the triton-extractable antigen; rather, penicillin treatment leads to an excessive production (R. Morrison and H. Caldwell, personal communication, 1987). Antichlamydial antibodies rapidly appear in the tears of those with trachoma and are capable of neutralizing chlamydia. However, despite the presence of high titters of tear antibodies, chlamydia can routinely be isolated from the conjunctiva, and it seems unlikely that antibodies are responsible for the observed phenomenon. Recently, gamma interferon has been shown to inhibit the growth of chlamydia. It is possible that this or other cytokines could lead to the arrest of chlamydial replication with subsequent antigen release. This suggests that the immune response may be enough to block chlamydial replication but not enough to completely eliminate infection; and clearly, the immune response is not capable of preventing future exposure to new inoculations of infectious organisms even if it were capable of preventing the establishment of new episodes of infection. Hence, antigenic products could be released by either persistent intracellular organisms or freshly inoculated but rapidly neutralized organisms. These antigens could further enhance the immune response. However, the immunopathogenetic mechanisms still require elucidation, and those patients with severe disease in the absence of demonstrable chlamydia form an important group for further study.

Finally, it was of interest to examine the serotype of chlamydia associated with trachoma in this part of Africa. The finding of serovars A and B is consistent with reports from other areas. Although the members of only one family were typed, all shared the same serovar; and similar findings have been reported from Saudi Arabia and Taiwan. The finding of a single serotype within a family supports the contention that intrafamily transmission is of prime importance. There was no correlation between serovar and disease severity. The increased frequency of serovar B in older people is unexplained but was also reported in Saudi Arabia.

This study demonstrated the advantages of the recently developed DFA method over chlamydial culture for use in trachoma field studies. The simplicity of DFA, particularly the lack of the requirement for a cold chain, greatly facilitates field work. We have confirmed the presence of chlamydial infection with the serovars A and B in this population in Central Tanzania. The identification of groups of people with proven infection in the absence of clinical disease and those with clinical disease in the absence of demonstrable agent provides important targets for further investigations on the dynamics of infection within the family transmission unit.

Key words: trachoma, laboratory diagnosis, culture, direct fluorescent antibody cytology, clinical grading

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