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Tubal infertility in the Gambia: chlamydial and gonococcal serology in women with tubal occlusion compared with pregnant controls

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Levels of immunoglobulin G (IgG) antibodies to Chlamydia trachomatis *and* Neisseria gonorrhoeae *were determined by enzyme-linked immunosorbent assay (ELISA) in 37 infertile Gambian women with bilateral fallopian tube occlusion and in 37 pregnant controls matched for age, ethnic group, and domicile. The infertile women had a significantly higher prevalence of antibodies to* C. trachomatis *serotype L1 (*$P = 0.01$*) and to purified* N. gonorrhoeae *pili, outer membrane, and lipopolysaccharide antigens (*$P < 0.01$ *in each case). Serological studies of immunoglobulin A (IgA) antibodies to* C. trachomatis *were less sensitive than the IgG studies in discriminating between the pregnant and infertile groups, suggesting that much of the infertility due to chlamydiae was the result of past rather than current infection. The data suggest that both* C. trachomatis *and* N. gonorrhoeae *are important causes of infertility due to tubal pathology in Gambian women.*

Pelvic inflammatory disease and its sequelae are extremely common in many parts of Africa (*1, 2*), and tubal occlusion following the disease is probably the most common cause of infertility among African women (*3-6*); in some areas more than 20% of women are involuntarily childless (*2*). Since the tuberculous form of the disease is uncommon in Africa (*7*), the vast majority of cases are thought to be due to ascending infection, and in the past this was assumed to be predominantly gonococcal (*2*). Recent reports suggest, however, that genital chlamydial infection may be as common as gonorrhoea among African women (*8, 9*). *Chlamydia trachomatis* is responsible for many cases of pelvic inflammatory disease in industrial countries (*10*) and is more likely than gonococcal disease to lead to infertility (*11*).

Both chlamydial and gonococcal salpingitis produce high titres of persistent circulating IgG antibody (*10*), representing past and current infection. To establish the relative importance of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in the etiology of tubal infertility in the Gambia, we compared antibody levels against these organisms in sera from patients with bilateral tubal occlusion and from matched pregnant controls, as determined by enzyme-linked immunosorbent assay (ELISA) using a method that takes into account both the concentration and relative avidity of the antibodies (*12*).

MATERIALS AND METHODS

The 37 infertile patients studied had all attended the gynaecology clinic in the government hospital in Banjul because of infertility; 28 were resident in Banjul or a nearby town and 9 were from rural areas. All major tribal groups were represented in approximately the proportions in which they occur in the general population. Thirteen of the patients were suffering from primary infertility, never having delivered a living or stillborn baby, while 24 were suffering from secondary infertility, having delivered babies in the past but having failed to conceive subsequently for varying periods (2-13 years; on average, 7.3 years). All patients had bilateral occlusion of the fallopian tubes, demonstrated in 29 cases by laparotomy and in the remaining 8 by hysterosalpingography.

As controls, 37 women were selected who were

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Table 1. Age, domicile, and ethnic background of patients (infertile) and controls (fertile)

Subjects	No.	Mean age (years)	Domicile		Ethnic group						
			Rural	Urban	Mandinka	Wollof	Jola	Fula	Serer	Creole	Other
Patients	37	29	9	28	11	8	8	4	2	2	2
Controls	37	29	9	28	11	8	6	5	5	1	1

attending antenatal clinics. The controls were carefully matched with the infertile patients for age, ethnic group, and place of residence (Table 1). Serum samples were stored at −20 °C until dispatch by air-freight to Southampton University Medical School for testing.

Serology

The serotypes of *C. trachomatis* and *N. gonorrhoeae* prevalent in the Gambia have not yet been identified; however, *C. trachomatis* serotype L1 was chosen as test antigen because of its broad reactivity with a wide range of antibodies to *C. trachomatis*. Similarly, α-pili from *N. gonorrhoeae* strain P9-2 contain immunodeterminants common to all gonococcal pili, and outer membrane antigen from *N. gonorrhoeae* strain P9-13 contains both proteins I and II. The lipopolysaccharide content of this outer membrane preparation was approximately 30% by dry weight. The properties of these antigens and of gonococcal lipopolysaccharide have been described previously (*13–15*).

Levels of antibodies were determined by ELISA using conventional techniques. For the analysis, use was made of antigens suspended in a 0.1 mol/l solution of carbonate-bicarbonate buffer (pH 9.6) at protein concentrations of 10 mg/l for *Chlamydia*, 1 mg/l for gonococcal pili and outer membrane, or 1 mg dry weight per litre for gonococcal lipopolysaccharide. Concentrations of antigens greater than these levels did not significantly increase the sensitivity of the method. Lipopolysaccharide was coated overnight at 37 °C on to activated polystyrene ELISA plates.[a] Gonococcal antigens and chlamydiae were coated overnight at 37 °C and 4 °C, respectively, on to poly-(vinyl chloride) ELISA trays.[a] Tripling dilutions of sera from 1:100 to 1:218 700 were prepared in a 0.05 mol/l solution of Tris buffer (pH 7.4) containing 1% (v/v) normal goat serum. The second antibody was a peroxidase-conjugated, heavy-chain-specific, rabbit anti-human IgG or IgA,[b] and the substrate was a 0.15 mol/l solution of tetramethylbenzidine in a 0.1 mol/l solution of sodium acetate (pH 6) and aqueous 0.0003% (v/v) H_2O_2. The absorbance was determined using a spectrophotometer, with a background absorbance ($\lambda = 450$ nm) of 0.01–0.04 units in the absence of human serum.

Analysis of data

For ease of computation, the tripling serum dilutions (1:100, 1:300, 1:900, etc.) were transformed to an integral $\log_3$-scale. After subtraction of the background reading, the value of the absorbance was used to calculate the area under the titration curve using Simpson's rule by adaptation of a BASIC computer program (*16*). Statistical analysis of the data was performed on a Honeywell 6080 mainframe computer using the software packages Minitab[c] and SPSS-X.[d]

RESULTS

Most sera contained low levels of "natural" antibody of low titre and avidity that reacted with the ELISA test antigens. The area under the titration curve for each serum provided much better discrimination than that provided by observation of the end-point when titration was carried out against these weakly reactive antibodies. It avoided the need for arbitrary definition of end-point and made full use of all titration data. Inevitably there was a high prevalence of IgG antibody in the fertile women of the control group because of the high incidence of cervical chlamydial and gonococcal infection (*8*), the presence of chlamydial antibody as a result of trachoma, and the long-term persistence of the antibodies generated (*10, 17*). This was evident when the frequency distribution of IgG antibody, determined by ELISA, was plotted for subjects and controls for four test antigens (Fig. 1).

The distributions plus the results of the assay on control sera permitted a distinction to be made between positive and negative test sera. Titres of positive sera ranged from 1:2700 to 1:72 900 for *Chlamydia*, gonococcal pili, or outer membrane and

[a] Flow Laboratories, Irvine, Scotland.

[b] Dako-Pats, Copenhagen, Denmark.

[c] From Pennsylvania State University, University Park, PA 16802, USA.

[d] SPSS Inc., Chicago, IL 60611, USA.

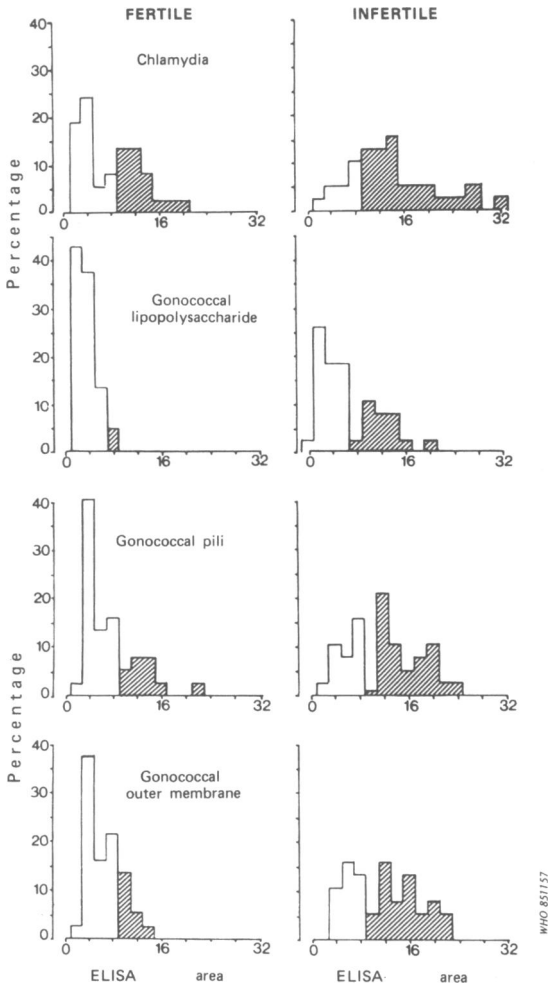

Fig. 1. Antibody response to the 4 antigens shown by the fertile (controls) and infertile Gambian women surveyed. The graphs show the percentage of individuals against antibody response plotted as the area under the ELISA titration curve (see text). Positive sera are shown hatched.

from 1:300 to 1:2700 for gonococcal lipopolysaccharide. The highest positive titres were recorded among the infertile women, reflecting the sensitivity of this method compared with conventional microimmunofluorescence. The prevalence of IgG antibodies to chlamydial and all gonococcal antigens was significantly higher among infertile women than it was among pregnant women ($P \leqslant 0.01$), as indicated by a χ^2 test with Yates correction (Table 2). Women with primary infertility had a statistically insignificant reduction in prevalence of antibody to *Chlamydia* compared with women who had secondary infertility ($P = 0.26$) but an increased prevalence of antibody to *N. gonorrhoeae* pili ($P = 0.01$) or outer membrane ($P = 0.05$).

Comparison of the mean level of antibody in positive sera among the infertile women and controls indicated no significant difference in the level of antibodies to gonococcal pili using a one-tail, two-sample Student's *t* test, but the levels of antibodies to gonococcal outer membrane and *Chlamydia* were higher in the infertile group (Table 3). Among the infertile women, 24 sera were positive for antibodies to gonococcal pili and, of these, 21 sera were also positive for antibody to gonococcal outer membrane, while 12 were positive for antibody to lipopolysaccharide. For the fertile women, 8 sera were positive for gonococcal outer membrane antibodies and, of these, only 1 lacked antibodies to pili.

When the prevalence of antibodies to both gonococci and chlamydiae was examined in the infertile women, 18 sera that were positive for chlamydial antibody also had antibodies to gonococcal pili. However, 7 sera that were positive for antibodies to *C. trachomatis* lacked antibodies to gonococcal pili, and a further 6 sera possessed antibodies to pili but lacked antibodies to *C. trachomatis*, confirming that gonococcal and chlamydial infections were acquired independently.

In total, 33 of 37 (89%) infertile women had antibodies to either gonococcal antigens or *Chlamydia* or both, compared with 17 of 37 (46%) of the matched, pregnant controls.

DISCUSSION

The results indicate that infertile Gambian women with tubal pathology have a higher prevalence and higher levels of circulating IgG antibody against *C. trachomatis* and *N. gonorrhoeae* than do pregnant controls and that these differences are statistically highly significant. This suggests that these women have suffered more frequent, more prolonged, or more severe infections than have the pregnant controls, and we conclude that *C. trachomatis* and/or *N. gonorrhoeae* is likely to have caused the tubal pathology in a high proportion of cases.

The proportion of cases of pelvic inflammatory disease from which *N. gonorrhoeae* was isolated has been reported in several urban African gynaecology clinics and varies from 4% to 43% (*18–21*). Since the symptoms of gonococcal pelvic inflammatory disease are more severe than those of non-gonococcal infection (*22*), hospital-based studies probably overestimate the proportion of gonococcal disease in the community.

Table 2. Prevalence of IgG antibodies, as determined by ELISA, to the antigens indicated among pregnant and infertile women in the Gambia

IgG antibodies to:	IgG-positive sera among: Pregnant women	Women with primary infertility	Women with secondary infertility	All infertile women	Significance[a] χ^2	P
C. trachomatis L1	13/37 (35)[b]	8/13 (62)	17/24 (71)	25/37 (68)	6.545	0.010
N. gonorrhoeae:						
α-pili	10/37 (27)	12/13 (92)	12/24 (50)	24/37 (65)	9.310	0.002
Outer membrane	8/37 (22)	10/13 (77)	13/24 (54)	23/37 (62)	10.881	0.001
Both pili and outer membrane	8/37 (22)	10/13 (77)	11/24 (46)	21/37 (57)	9.830	0.002
Lipopolysaccharide	2/37 (5)	5/13 (38)	7/24 (29)	12/37 (32)	7.136	0.008

[a] Pregnant versus infertile women.
[b] Figures in parentheses are percentages.

Table 3. Mean IgG antibody response, as determined by ELISA, among pregnant and infertile women with positive antibodies to gonococcal and chlamydial antigens; a two-tail Student's *t* test was used to examine the significance of differences in mean antibody level

IgG antibody to:	Mean antibody response in IgG-positive sera among: Pregnant women	Women with primary infertility	Women with secondary infertility	All infertile women	Significance[a] P
C. trachomatis L1	13.4 (13)[b]	17.8 (8)	17.1 (17)	17.6 (25)	0.0047
N. gonorrhoeae:					
α-pili	13.7 (10)	14.5 (12)	14.2 (12)	15.3 (24)	0.1310[c]
Outer membrane	11.2 (8)	14.6 (10)	14.6 (13)	15.1 (23)	0.0002
Lipopolysaccharide	8.4 (2)	10.2 (5)	13.2 (7)	12.8 (12)	–

[a] Pregnant versus infertile women.
[b] Figures in parentheses indicate the number of women in each group.
[c] Not statistically significant.

In the only other study of chlamydial serology in infertile African women, the prevalence of antibody to *C. trachomatis* in patients in Johannesburg with tubal pathology was 87%, compared with 38% in pregnant controls, as indicated by microimmunofluorescence (*9*). In developed countries several serological studies have also shown significantly higher titres of circulating antibody to *C. trachomatis* in women with tubal infertility than in controls with normal fallopian tubes (*10, 22*). There have been few studies of gonococcal antibodies in infertile women, even though gonococcal pili are the basis of a sensitive and specific test for gonococcal antibody (*17, 23*), and outer membrane preparations from gonococci permit the detection of antibodies to protein I, protein II, or LPS (*15*). In Sweden chlamydiae, rather than gonococci, are the main cause of acute salpingitis (*10*), whereas in the Gambia our findings suggest that both organisms are important causes of infertility following salpingitis. This difference, if true of a wider geographical area, probably reflects the greater likelihood that gonococcal infections would be treated at an earlier stage in Europe.

The overall prevalence of female infertility in the Gambia is not known. However, in two rural villages where accurate records of births and deaths have been kept since 1951 (*24*), the prevalences of primary infertility are 3% and 5%, and those of secondary infertility, defined as failure to bear children after the age of 30, are 13% and 19%. Secondary infertility may be a sequela of postpartum sepsis, which is commonly observed at hospitals and clinics in the Gambia and which, given the association between cervical chlamydial infection and endometritis (*25*), may be due in part to chlamydiae. The present study indicates that there was a significant increase in the

prevalence of antibody to gonococcal pili or outer membrane in women with primary infertility, accompanied by a corresponding but statistically insignificant increase in the prevalence of chlamydial antibody in women with secondary infertility. These results imply that gonococci may be a more important cause of primary than of secondary infertility in the Gambia than are chlamydiae, but a bigger study sample would have to be analysed to establish this.

We conclude that both *C. trachomatis* and *N. gonorrhoeae* are important causes of tubal pathology in Gambian women. This reflects the similar prevalence of positive cultures for chlamydiae and gonococci, 6.9% and 6.7%, respectively, reported in a separate study of pregnant women in Bakau, in the Gambia (*26*). In that study the prevalence of chlamydial IgG antibody in sera from pregnant women determined by microimmunofluorescence was similar to the results reported here. Other workers have also found a high correlation between the results of ELISA and microimmunofluorescence tests for chlamydial antibody (*27–29*). The ELISA method was able to discriminate infection in the infertile group in the presence of a relatively high background level of infection in the antenatal controls. Compared with microimmunofluorescence, ELISA is cheap, lends itself more readily to quantification, and is much more readily adapted to screening large numbers of sera for chlamydial antibody.

IgA antibodies have recently been reported to be of particular value in the serological diagnosis of acute chlamydial infections by the ELISA method (*29*). In the present study 18 out of 37 infertile patients had IgA antibody to *C. trachomatis* serotype L1 compared with 11 out of 37 antenatal controls. The lower prevalence of IgA antibody among the infertile women compared with that of IgG indicates, however, that many of these women had antibody to past rather than to current chlamydial infection. The existence of sera that are positive for chlamydiae in patients with suspected acute salpingitis indicates that further investigation is required, and this should include attempts to isolate chlamydiae from the lower genital tract, although the presence of local antibody may impair this (*30*). It remains to be determined whether serological studies by ELISA for the presence of chlamydiae or gonococci will be effective in predicting "at risk" individuals among gynaecology patients *before* they develop tubal dysfunction. In addition, studies are needed to establish whether both *C. trachomatis* and *N. gonorrhoeae* are important causes of tubal pathology in other countries of Africa, including rural as well as urban areas. Should this prove to be the case, the long courses of antibiotics needed to treat genital chlamydial infection and the spread of penicillinase-producing gonococci indicate that immediate prospects of controlling infertility in Africa due to these infections are bleak.

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RÉSUMÉ

STÉRILITÉ TUBAIRE EN GAMBIE: SÉROLOGIE DES INFECTIONS À CHLAMYDIA ET À GONOCOQUES CHEZ LES FEMMES AVEC OCCLUSION TUBAIRE ET CHEZ DES TÉMOINS ENCEINTES

Les sérums de 37 femmes gambiennes stériles ayant des lésions des trompes de Fallope et de 37 témoins enceintes appareillées pour l'âge, la tribu et le domicile ont été examinés par une technique immunoenzymatique (ELISA) pour rechercher la présence d'anticorps du type IgG envers *Chlamydia trachomatis* LGV L1 et les pili, la membrane externe ou les lipopolysaccharides purifiés de *Neisseria gonorrhoeae* P9. Le rapport de la densité optique à λ = 450 nm à la dilution du sérum a été utilisé pour calculer l'aire de la zone située sous la courbe de titrage. Cette méthode est préférable à l'utilisation du point final du titrage parce qu'elle fait usage de toutes les données du titrage et permet une meilleure discrimination des anticorps croisants à faible avidité. Chez les femmes stériles, on a observé une prévalence nettement plus élevée des anticorps IgG envers le sérotype L1 de *C. trachomatis* ($P = 0{,}01$) ainsi que des IgG dirigés contre tous les antigènes gonococciques ($P < 0{,}01$ dans chaque cas).

Chez les malades et les témoins, les distributions de fréquence de l'aire obtenue pour les anticorps IgG par la méthode ELISA ont été établies pour les quatre antigènes d'épreuve, ce qui a permis de distinguer les sérums positifs des sérums négatifs. Le taux moyen d'anticorps IgG chez les malades stériles séropositives était plus élevé vis-à-vis de la

membrane externe des chlamydia et des gonocoques, mais il n'y avait pas de différence significative du taux d'anticorps envers les pili gonococciques. Chez les femmes enceintes et celles qui étaient stériles et séropositives, il y avait une corrélation étroite entre les anticorps IgG envers les pili gonococciques et les anticorps envers la membrane externe. Cependant, il est clair qu'en Gambie les infections à gonocoques et à chlamydia peuvent être acquises ensemble ou séparément. Les malades stériles porteuses d'anticorps IgG envers *C. trachomatis* L1 manquaient fréquemment de taux décelables d'anticorps IgA, ce qui fait penser que dans cette population la plupart des anticorps étaient dus à des infections à chlamydia antérieures plutôt qu'actuelles. Les taux plus élevés d'anticorps IgG envers *C. trachomatis* et *N. gonorrhoeae* chez les femmes stériles indiquaient qu'elles souffraient d'infections plus fréquentes, plus prolongées ou plus sévères que les femmes témoins enceintes. Les résultats semblent indiquer qu'en Gambie et peut-être dans d'autres pays de l'Afrique occidentale, *C. trachomatis* et *N. gonorrhoeae* sont des causes importantes de dysfonctionnement des trompes de Fallope pouvant conduire à la stérilité. Ceci s'oppose à la situation qui existe en Europe où *C. trachomatis* est considéré comme une cause plus importante de stérilité que *N. gonorrhoeae*.

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