Screening for toxoplasmosis in pregnancy

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SUMMARY The prevalence of antibody against *Toxoplasma gondi* in a population of 715 pregnant women has been evaluated by two methods: indirect haemagglutination antibody (IHA) and indirect fluorescent antibody (IFA) tests and all positive sera were checked by the dye test. Five hundred of the study population were questioned on diet and on animal contact to elucidate a possible relation to the prevalence of antibody.

Results are expressed in international units (IU) of antibody against *T gondi*. Of the 715 sera, 171 were positive by IHA and 173 by IFA. One hundred and sixty-seven sera were positive by both tests; ninety-eight (58%) correlating exactly, as to the concentration of antibody. The ten sera which were not positive by both tests all had detectable antibody at the minimum concentration only (12 IU). The dye test confirmed all sera positive by both tests with the exception of three. It also confirmed one of four sera positive by IHA antibody alone and two of six positive by IFA alone. All sera that proved dye test-negative had low antibody concentrations (12 IU) by IHA or IFA. The IHA test, which is commercially available in kit form, would be suitable for use as a screening test during pregnancy.

The estimated annual rate of antibody acquisition over the age range 16-40 years is 1.2% per annum with the highest rate in the 36-40 age group (2.5% per annum) and the lowest in the 26-30 age group (0.4% per annum). The clinical history was not significantly different between those with and those without antibody against *T gondi* but significantly more women in the 36-40 age group had a history of animal contact than those in the 26-30 age group. No conclusive evidence of recent or current infection was found.

Congenital toxoplasmosis can be severely damaging, even fatal, for an affected fetus and so, if the methodology is practicable it can be argued that screening for primary *T gondi* infection during pregnancy should be performed. The test of choice for detecting antibody against *T gondi* has been the dye test. This is a technically exacting and difficult method and a number of workers maintain that the indirect fluorescent antibody (IFA) method is an adequate substitute.¹ This latter method, however, is still time-consuming. By contrast the IHA method is quick and simple to perform. Furthermore, it is available in kit form. We undertook this study to compare the latter two tests as screening tests for toxoplasma antibody and to evaluate the role of a number of factors on the incidence of toxoplasma antibody.

Material and methods

TEST SAMPLES

Blood was collected from 715 pregnant women at the first visit to the antenatal clinic. The patients' ages ranged from 15 to 44 years. Five hundred of them had a second sample taken after parturition. Serum was separated, inactivated at 56°C for 30 min, and stored at -20°C until tested.

INDIRECT HAEMAGGLUTINATION TEST KIT

This was supplied by Burroughs Wellcome as the ToxHA test kit comprising: test cells coated with a soluble sonicate of *T gondi*, control cells to check for non-specific agglutination, diluent, and positive and negative control sera.

THE INDIRECT FLUORESCENT ANTIBODY TEST
Freeze-dried *T gondi* trophozoites were obtained

(Wellcome Reagents). They were reconstituted, spotted on a teflon-treated slide, and fixed. Patients' sera were tested at dilutions matching those for the IHA test. Selected sera were tested for toxoplasma-specific IgM.

WORLD HEALTH ORGANISATION TOXOPLASMA INTERNATIONAL REFERENCE SERUM FOR ANTIBODY

In order to read our results in international units, a reference serum was obtained from Statens Seruminstitut, Copenhagen, Denmark. This serum was included in each test run.

PREPARATION OF SERUM DILUTIONS

Fourfold sequential dilutions were initially prepared from 1/16 to 1/256 in a microtitration plate and used as a master dilution from which aliquots—that is, one drop, were taken and used in the IFA test. The remaining volumes were used in the HA test. This altered the volumes recommended by the manufacturer for the ToxHA test but did not appear to affect the results.

QUESTIONNAIRE

Five hundred subjects from whom post-delivery samples of blood were obtained were questioned on points relevant to the aetiology of toxoplasmosis. Answers to the questions are detailed in Table 5 (see later).

DYE TEST

We are grateful to Dr DG Fleck, in whose laboratory these tests were carried out.

Results

CORRELATION BETWEEN TESTS

Seven hundred and fifteen subjects were tested for the presence of antibody against *T gondi* by both tests. The numbers with detectable antibody by either test are shown in Table 1. Ten sera have detectable antibody by one test alone and the titres of all of these are at the minimal level (12 IU). Antibody concen-

tration detected by ToxHA test (IHA) appear to be higher than by the IFA technique and this was confirmed as a significant trend (Wilcoxon ranked sums test: $z = 3.25$ and $0.007 > p > 0.005$). The majority of sera with antibody had 50 IU or less by both tests and 98 of 167 sera positive by both tests had identical results (Table 2). The majority of the latter had 50 IU of antibody against *T gondi*. Larger differences are seen between the two tests in high titre sera (800 IU) than in lower titred sera.

Table 2 Numbers of subjects in whom antibody concentrations correspond in both tests

Test	No of subjects	Concentration of antibody (IU)			
		12	50	200	> 800
IHA and IFA	98 (100)	18 (18.4)	64 (65.3)	15 (15.3)	1 (1.0)

Percentages are shown in parentheses.

IHA = indirect haemagglutination antibody.

IFA = indirect fluorescent antibody.

EFFECT OF AGE ON PRESENCE OF ANTIBODY TO *T GONDI*

The date of birth was not available for three of the population of 715 under study. Table 3 shows the age distribution and the occurrence of antibody-positive individuals in each group. The incidence of those with antibody increases with age from 16-40 years of age with an apparent reduction over 40 years of age. If this latter group is excluded on the grounds of the small size of the sample (1.5% of the total population) then the highest estimated annual antibody acquisition rate is in the 36-40 age group and the lowest in the 26-30 age group. Application of the χ^2 test using a fourfold table to these two groups has shown that there is a significant difference in the incidence of antibody in each ($\chi^2 = 7.6$; $0.01 < p < 0.001$).

HIGH TITRE SERA

High titre sera (>800 IU) are presented by age group in Table 4. This shows that the greatest concentration occurs in the age group with the highest estimated annual rate of antibody acquisition.

TOXOPLASMA-SPECIFIC IgM

All high titre sera (>800 IU) were checked for toxoplasma-specific IgM. None was found positive.

DYE TEST

This was carried out at a separate laboratory and confirmed all sera positive by IHA and IFA, with the exception of three. Of the ten sera positive by IFA or IHA alone, one IHA-positive and two IFA-positive were confirmed by the dye test. In all cases where the

Table 1 Antibody against *Toxoplasma gondi* detected by IFA and IHA in 173 subjects

Test	No of subjects	Concentration of antibody (IU)			
		12	50	200	> 800
IHA	167 (171)	23 (27)	87	46	11
IFA	167 (173)	35 (41)	98	31	3

Figures in parentheses include the ten sera positive by one test only.

IHA = indirect haemagglutination antibody.

IFA = indirect fluorescent antibody.

dye test failed to confirm a positive IHA or IFA result the concentration of antibody did not exceed 12 IU.

RESULTS OF QUESTIONNAIRE

Table 5 shows the answers obtained from the whole

population, those with and without antibody against *T gondi* and from the age groups with the highest and the lowest antibody acquisition rates. A fourfold table to ascertain chi-squared (χ^2) gave the following results: no difference of great significance in the clinical history could be discerned between the sero-

Table 3 *Antibody against Toxoplasma gondi in pregnant women grouped in five year age spans*

Age	No in group	% of study population	No positive	% positive	Estimated seroconversion rate per annum (%)
16-20	61	8.6	10	16.4	—
21-25	152	21.2	32	19.7	0.7
26-30	286	40.0	62	21.7	0.4
31-35	154	21.5	42	27.3	1.1
36-40	48	6.7	19	39.6	2.5
40+	11	1.5	1	9.1	—
Date of birth not available	3	0.4	1	—	—
Total sera	715	99.9	167	23.3	1.2*

*Subjects ≤ 40 yr.

Table 4 *High titre sera (800 IU)*

Age	No of high titre sera in each group	% of high titre sera in each group	Estimated rate of seroconversion per annum (%)
16-20	1	1.6	—
21-25	3	2.0	0.7
26-30	2	0.7	0.4
31-35	3	1.9	1.1
36-40	2	4.2	2.5
40+	0	—	—

Table 5 *Results of a questionnaire concerning the aetiology of toxoplasmosis*

Questions		All patients (500)	Subjects with detectable antibody	Subjects without detectable antibody	Age group* with lowest annual acquisition rate of antibody	Age group† with highest annual acquisition rate of antibody
Do you eat undercooked or raw meat?	Yes	162 (32.5)	39 (33.6)	124 (32.2)	62 (33.3)	12 (44.3)
	No	338 (67.5)	76 (66.4)	260 (67.8)	125 (66.7)	15 (55.7)
Were you born in the UK?	Yes	370 (74)	80 (70)	268 (69.8)	139 (74.5)	17 (63)
	No	130 (26)	35 (30)	116 (30.2)	48 (25.5)	10 (37)
Do you have contact with farm animals?	Direct	97 (19.4)	25 (21.2)	73 (18.9)	37 (19.8)	5 (18.5)
	Indirect	79 (15.8)	21 (18.6)	57 (14.9)	30 (16.0)	10 (37)
	No	324 (64.8)	69 (60.2)	254 (66.1)	120 (64.2)	12 (44.4)
Do you like cats?	Yes	294 (58.5)	70 (61.3)	223 (58)	106 (56.8)	19 (70.4)
	No	206 (41.2)	45 (38.7)	161 (42.1)	81 (43.2)	8 (29.6)
Do you keep cats?	Yes	168 (33.7)	37 (32.2)	132 (34.2)	58 (31.1)	16 (59.2)
	No	332 (66.3)	78 (67.8)	252 (65.7)	129 (68.9)	11 (40.8)
Do you have contact with cats?	Frequent	252 (50.4)	50 (43.4)	203 (52.8)	94 (50)	15 (55.5)
	Infrequent	76 (15.2)	23 (20.3)	52 (13.6)	18 (9.9)	3 (11.1)
	No	172 (34.3)	42 (36.3)	128 (33.6)	75 (40.1)	9 (33.3)
Does your next door neighbour have a cat?	Yes	265 (52.9)	61 (53.2)	203 (52.8)	104 (55.5)	16 (59.2)
	No	235 (47.0)	54 (46.8)	181 (47.2)	83 (44.5)	11 (40.8)
Do you like dogs?	Yes	408 (81.7)	87 (76.4)	313 (81.7)	145 (77.4)	23 (85.1)
	No	92 (18.3)	28 (23.6)	71 (18.3)	42 (22.6)	4 (14.8)
Do you come in contact with dogs?	Frequent	211 (42.2)	44 (38.0)	167 (43.5)	79 (42.3)	9 (33.3)
	Infrequent	83 (16.6)	23 (20.4)	59 (15.3)	32 (16.9)	7 (25.9)
	No	206 (41.3)	48 (41.6)	158 (41.2)	76 (40.7)	11 (40.8)
Does your next door neighbour have a dog?	Yes	211 (42.1)	51 (43.9)	159 (41.3)	81 (43.1)	15 (55.7)
	No	289 (57.9)	64 (56.1)	225 (58.7)	106 (56.9)	12 (44.3)

Percentages in parentheses.

*Age group = 26-30 yr.

†Age group = 36-40 yr.

positive and seronegative subjects but a highly significant difference existed between the two age-groups on the question of keeping cats ($\chi^2 = 8.3$ and $0.01 > p > 0.001$) and less significantly ($\chi^2 = 3.88$ and $0.05 > p > 0.02$) on the question of contact with farm animals.

Discussion

The identification of those at risk and the prevention of intrauterine infections by *T. gondii* is important since the treatment of both maternal and congenital toxoplasmosis available at present is inadequate.

Congenital infection by *T. gondii* can occur after primary infection during pregnancy. The possibility of exception to this rule has been shown in chronically-infected mice,¹⁹ but if this occurs in humans, it is certainly very rare. Thus, the demonstration of antibodies, which indicate that contact with *T. gondii* preceded the current pregnancy, may allow a reasonable assumption that neither the child in utero nor any subsequent children are at risk from toxoplasmosis. The technical problems associated with the dye test have led workers to examine the less-exacting IFA method. Overall, in adults, with rare exceptions, the IFA test agrees well with the dye test. Published levels of correlation between the dye test and the IHA or between the latter and the IFA technique are more widely varied.²

The IHA method can give rise to false-negative results in neonatology.³ However, the reasons for testing are largely to determine if infection has occurred in utero and this is possible by prenatal screening of pregnant women. We think that both the dye test and the IFA method are too cumbersome for this purpose. The IHA method, particularly now available in kit form, would be more suitable.

In this study there was good correlation between both IHA and IFA tests in distinguishing positive from negative sera in all but ten low titre sera (Table 1). Six were positive by IFA alone and four were positive by IHA alone. The dye test confirmed two of the former and one of the latter. These results do not show one test as superior to the other in detecting low concentrations of antibody against *T. gondii* when checked by the dye test.

Other workers using these methods have obtained results which differed from our own both in reliability and the antibody pattern seen.^{4,5} Our work showed for example, higher results for the IHA test than the IFA test (Table 1). However, other work suggests that this is the expected pattern.⁶

Although not used in the studies mentioned above, it seems important to include a standard serum in any investigation of this kind. In our study an international reference serum was used (recommended by

the WHO since 1968), for it is by the use of such controls that comparability between studies will be more easily attained.

Table 3 shows that the lowest rate of antibody acquisition occurred in the 26-30 yr age-group. This agrees with the observations of Kimball *et al.*⁷ and Viens *et al.*⁸ but differs from the observations of Ruoss and Bourne.⁹ The age-group showing the highest rate of antibody acquisition differed in all the studies mentioned above. It is important to define this age-group, for the higher the rate of antibody acquisition (primary infection) the greater will be the risk of congenital toxoplasmosis.⁷

From our study, the lowest incidence of congenital toxoplasmosis would be expected in children born to women aged 26-30 (40% of the study population) and the highest incidence in those born to women aged 36-40 (67%). Ruoss and Bourne⁹ found their highest estimated seroconversion rate in the 20-25 age-group and a stable low rate in the two groups 30-35 and 36-40, but the pregnant population they studied was, on the whole, much younger than our own; 47.5% were between 15 and 20 years of age. This age distribution is markedly different from that generally seen in antenatal clinics in England and Wales and differs from that seen in this study population where the age distribution closely matched that of mothers of children born in 1976.¹⁰

We found 11 patients with a high titre of toxoplasma antibody (≥ 800 IU by either test), but we could not demonstrate toxoplasma-specific IgM in any. Karim and Ludlam⁶ presented data on antibody detected by the IFA and IHA tests which showed that the pattern of response to the antigens allows a crude dating of infection by *T. gondii*. Using their criteria, two of our patients had an antibody pattern which suggested infection in the last six months, but our inability to demonstrate specific IgM leaves the evidence for recent infection unsupported. There was no evidence of congenital toxoplasmosis in their offspring. The titre of antibody against *T. gondii* has been proposed as a useful way of dating infection.¹¹ While we have reservations on this proposal it does seem likely that the more recent infections will have higher titres than less recent infections. In Table 4 we set out high titre sera according to age; and this shows that the highest proportion of high titre sera occur in the age-group with the highest estimated rate of annual acquisition of antibody. The numbers are too small for statistical analysis so any conclusions must be tentative but the trend seems to support the observation that "recent" infection occurs most frequently in one of the smallest age-groups (Table 3).

The questionnaire produced only one significant finding; the group with the highest rate of acquisition of antibody (36-40 years) showed the highest

incidence of keeping cats. Ownership of cats was not a feature distinguishing antibody-positive and antibody-negative individuals overall. It may be possible to explain this apparent discrepancy by reference to the persistence of antibody against *T. gondii*. In the high antibody acquisition group it may be assumed that more recent contact with cats exists and so is more likely to be remembered than contact in early life.

The rate of antibody acquisition in our study population was 1.2% per annum. This is higher than that usually seen in the UK where a range of 0.5-1.0% per annum is reported. This is lower than the 1.75% per annum experienced in France.¹² As would be expected, the French rates of congenital infection are higher at 1/2000¹³ than the British rate of 1/16 000.¹⁴ However, the order of magnitude is unexpectedly high, for an increase of less than double in sero-conversion rates during the child-bearing period apparently results in an eightfold increase in congenital disease. This suggests that there are other factors in France when compared with UK which influence the rates of maternal transmission to the fetus. Two of possible importance are the initial dose of parasite,¹⁵ and the influence of maternal antibody on the fetal infection.¹⁶ It seems likely that the lower the parasite dose and thus the less rapid and severe the ensuing parasitaemia, the greater the ability of the immune system to control the infection. It is generally suggested that the high rate of infection seen in France is due to ingestion of undercooked infected meat and strong supporting evidence for this has been provided.¹⁷

In this study the only factor apparently associated with increased incidence of the disease was contact with cats—ingestion of undercooked meat had no significance. The oocyst in feline infection contains only eight infective units whereas the tissue cyst found in infected meat can contain several thousand infective parasites. Furthermore, in the latter case, repeated contact is more probable.

Low dose infection could explain why the offspring of the seven mothers with presumed primary infection described by Ruoss and Bourne were spared congenital infection.⁹ This contrasts with the position in France where, when primary toxoplasmosis occurs during pregnancy, a congenital infection rate of 33-39% is seen.¹⁸ However, the infecting dose is probably only one factor. As has been mentioned, a link exists between seroconversion rates and the incidence of congenital toxoplasmosis. In our study the highest extrapolated antibody acquisition rate occurred in one of the smaller groups and the lowest rate in the largest group.

We therefore finally propose, and there is evidence for both points in this study, that the reason for the

low rate of clinically apparent congenital toxoplasmosis seen in this country may be due first to the majority of primary infections arising from a low parasite dose (ingestion of oocysts), and secondly that during the child-bearing years, the group with the highest incidence of primary infection is less than 10% of the pregnant population.

Toxoplasmosis is predominantly an acquired disease. Assessment of immunity by the ToxHA test kit and an IFA method gave results close enough for us to recommend the IHA method as a screening test in pregnancy. The pattern of antibody acquisition was irregular and showed high and low rates in different age groups. Our study suggests that women of 36-40 yr have a higher risk of infection with *T. gondii* than women aged between 15-35 yr and that this risk may be associated with keeping cats as pets.

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