J. Hyg., Camb. (1980), 85, 165
Printed in Great Britain

Prevalence of toxoplasma antibodies according to age with comments on the risk of prenatal infection

By J. van der Veen and M. F. Polak
Laboratory for Medical Microbiology, University of Nijmegen, Nijmegen, The Netherlands

(*Received* 19 *December* 1978)

SUMMARY

Sera from 1661 persons in 12 age groups from 0 to 79 years were titrated for toxoplasma antibodies in the indirect immunofluorescence test. The sera were collected from patients with symptoms suggestive of acute, mainly respiratory, viral infections. After the first year of life, the prevalence of antibodies started to rise, reaching 59% between 40 and 79 years of age. From the prevalence of antibodies in different age groups the annual infection risk, i.e. the risk of a non-immune person acquiring toxoplasma infection, was estimated for successive age periods. The estimated annual infection risk increased from 0·5% in early childhood to 3% during adolescence and early adult life.

Approximately 70–80% of females entered the age of reproduction without evidence of seroimmunity to toxoplasma. The risk of primary infection during pregnancy was estimated from the age distribution of parturient women in The Netherlands in 1975 and the age-specific incidence of primary infections, i.e. the incidence in the total population of susceptible and immune persons. This incidence of primary infection decreased from 1·62% per 9 months at the age of 17½–20 years to 0·37% at the age of 35–45 years. The incidence of primary infections in pregnant women was estimated to be 1·25%.

INTRODUCTION

It is generally accepted that, apart from the transplacental route of infection, humans may acquire toxoplasma infection by two routes: consumption of raw or undercooked meat which contains toxoplasma cysts and ingestion of sporulated oocysts from faeces shed by infected cats (Frenkel, 1973). In view of the variations in meat-eating habits and in the degree of exposure to infectious faeces of cats the prevalence of infection as shown by sero-epidemiological studies may differ widely between various countries and between areas within a country (Remington & Desmonts, 1976). The risk of congenital infection in a given population is not commensurate with the prevalence *per se* since it is restricted to the occurrence of *primary* infection during pregnancy. In areas of high infection rates during childhood and adolescence most of the females have been infected before the childbearing age. Under these circumstances the frequency of primary infection during

0022-1724/80/0141-1978 $01.00

pregnancy and, consequently, the fetal risk will be low. Low infection rates in younger persons and in adults of child-bearing age will have a similar outcome. The fetal risk will be relatively high in areas with a constant rate of infection, intermediate between 'low' and 'high', in children and adults.

In order to obtain information on toxoplasma infection risks at different ages, we examined sera from 1661 persons of various age groups for toxoplasma antibodies by the indirect immunofluorescence test. The sera were derived from patients with clinically suspected acute, mainly respiratory, viral infections. With the aid of this information estimates were made of the frequency of primary maternal infection and the associated risk of fetal infection.

MATERIALS AND METHODS

Sera were obtained from the diagnostic virus laboratory of the St Elisabeth Hospital in Tilburg, The Netherlands. They were collected between 1972 and 1976, and were stored at −20°C. One hundred sera from each age group (0–5 months, 6–11 months, 1–2, 3–4, 5–9, 10–19, 20–29, 30–39, 40–49, 50–59, 60–69 and 70–79 years), 50 from male and 50 from female patients, were allocated to this study on toxoplasma antibodies. Except for the requirement of a minimal quantity of 0·5 ml, further selection criteria were not applied.

A preliminary evaluation of the test results suggested that a great increase in seropositivity occurred in the 10–19 years age group. It was therefore deemed desirable to allocate additional sera from 10–19 years old persons to this study; 476 sera from the same source were collected for this purpose.

Sera were titrated in the indirect immunofluorescence (IF) test as described by van Nunen & van der Veen (1965). The dilution steps were twofold; the first dilution was 1/8. In order to cope with possible variations in the sensitivity of the IF test, equal numbers of sera per age group and sex were titrated on each day. In addition, two known positive sera and a negative serum were included. No differences in titre of the positive control sera were observed. The results of 15 sera are not accounted for because of doubtful immunofluorescence. Titres are expressed as reciprocal values of the highest dilution showing fluorescence.

For comparison, a reference toxoplasmosis serum containing 500 international units per ml was examined in the IF test. The serum was provided by Dr Siim, Copenhagen. The titre was 2048 in each of five separate tests.

From the prevalences of antibodies in successive age groups the estimated annual infection risk (k), i.e. the risk of a non-immune person acquiring infection, was calculated from

$$k = \left(\frac{\log_e P_0 - \log_e P_1}{t}\right)100,$$

where P_0 is the percentage of negative sera in the younger age group, P_1 is the percentage of negative sera in the older age group, e is the base of natural logarithms, and t is the interval (years) between the median ages of both age groups. Thus, k represents a percentage – that is, the number of persons acquiring infection per annum per 100 non-immune persons.

In applying this calculation we consider the observed percentage of negative sera in any given age group as an estimate of susceptibility (or non-immunity) at the median age of this group. An additional assumption is that the results of our transverse study can be used as a correct description of a longitudinal study; that is, we assume, when comparing infection risks in successive cohorts, that factors which imply a risk of becoming infected with toxoplasma such as consumption of raw meat, contact with cat faeces, and climate showed negligible variation in the past decades.

RESULTS

Table 1 shows the distribution of titres of sera from 1661 persons by age group. There were no significant differences in titre distribution between the four 10-years age groups between 40 and 79 years (titres higher than 256 combined, $\chi^2 = 10{\cdot}86$, D.F. 15, $P > 0{\cdot}7$). Therefore, the results for these groups were combined in two 20-year age groups.

Bimodal pattern of titre distribution

The titre distribution for the three age groups between 1 and 9 years was bimodal so that two subpopulations could be distinguished, one consisting of negative or very low titred sera and the other consisting of moderately high or high titred sera. Thus, none of the 300 children in these groups had titres of 32–128 whereas seven of them showed a titre of 256 or higher and 24 a titre of 16 or 8, the remaining being seronegative. This bimodal pattern of titre distribution was also observed in the six age groups above 10 years although it was less clear-cut. Titres of 32–128 that were absent in children between 1 and 9 years of age were present in older persons. Moreover, the proportion of these titres as well as that of titres of 8 and 16 increased with age in the groups between 10 and 39 years. After 40 years, the pattern of titre distribution did not change.

The bimodal pattern of the titre distribution asks for interpretation. One may speculate that a major part of the very low titres reflects non-specific reactions or reactions due to cross-reacting antigens whereas the other part results from infection with toxoplasma. In order to account for the difference in the bimodal pattern between the young and older age groups one might further postulate that the proportion of low titres of specific origin increases with age. On the basis of these postulates, we decided to consider all titres of 8 and 16 in the age groups between 1 and 29 years as negative as well as all titres of 8 in the 30–39 years age group, but only a part of titres of 16 in the 30–39 years age group and a part of titres of 8 and 16 in the 40–79 years age group. To determine the share of 'negative' titres in the two latter groups the ratio of frequencies of the titres < 8, 8, and 16 in the 10–29 years group, namely 100:21:8, was applied to the titres in the older age groups. Titres of 16 in the 30–39 years age group and titres of 8 and 16 in the 40–79 years age group in excess of the ratio were considered as positive. The titre distributions of 'negative' and 'positive' sera as calculated in this way and expressed as percentages are presented in Fig. 1.

Table 1. *IF-titre distributions expressed as percentages*

IF titre	Age group (years) and number of sera per age group									
	0–5/12	6–11/12	1–2	3–4	5–9	10–14	15–19	20–29	30–39	40–59	60–79
<8	100	100	100	100	100	295	273	100	100	196	197
8	81	98	93	90	86	69·5	58·2	46	34	31·6	31·5
16	1	1	5	6	9	14·6	11·7	11	9	13·3	9·6
32–64	6	1	—	2	2	4·7	6·2	3	12	12·8	12·7
128–256	5	—	—	—	—	2·7	6·2	10	18	15·3	17·8
512–1024	6	—	1	—	2	4·1	9·9	16	20	18·4	20·3
>2048	1	—	—	2	1	3·7	5·1	12	6	7·1	8·1
	—	—	1	—	—	0·7	2·6	2	1	1·5	—

https://doi.org/10.1017/S0022172400063191 Published online by Cambridge University Press

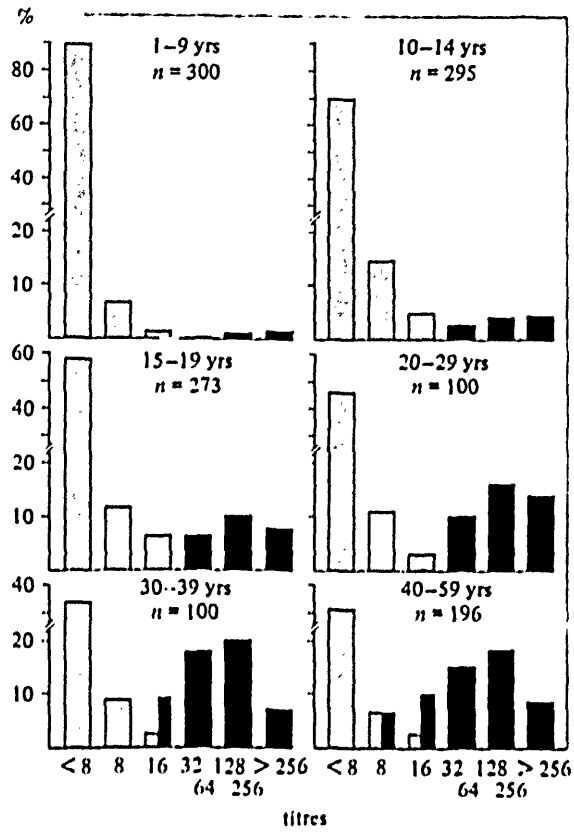

Fig. 1. Distribution of IF titres in six age groups expressed as percentages. Black columns represent sera considered as positive for toxoplasma antibodies.

Prevalence and titre of antibody at different ages

The prevalence and titres of antibodies in infants less than 6 months old were fairly high (Table 1). In contrast, only two children in the 6- to 11-months-old group had antibodies, and the titres were low. We interpret this as indicating that in most cases antibodies present in children under 6 months of age are of maternal origin and disappear from the circulation during the first year of life.

After the first year of life, the prevalence of antibodies started to rise and the frequency of positive sera (see preceding paragraph) reached a maximal value of 59 % in the age groups between 40 and 79 years (Table 2). The titre distribution shows a peak at titres of 128 and 256 for all age groups and a relatively high frequency of titres $\geqslant 2048$ in the 15–19 years age group (Table 1).

Infection risk by age

Direct comparison of prevalence rates by age is inadequate when the aim is to compare incidences of *primary* infections in successive age groups since it does not take into account the continuous decrease of susceptible persons with increasing age. Therefore, the infection risk, i.e. the risk of non-immune (seronegative)

https://doi.org/10.1017/S0022172400063191 Published online by Cambridge University Press

Table 2. *Negative sera per age group and annual infection risks between median ages of successive age groups*

Age group (years)	Negative sera*		Age period between medians of age groups (years)	Annual infection risk per age period (%)†
	Frequency	%		
3/4		100‡		
			3/4–7½	0·45
1–4	196/200	98		
			7½–12½	1·8
5–9	97/100	97		
			12½–17½	3·1
10–14	262/295	88·8		
			17½–25	3·2
15–19	208/273	76·2		
			25–35	2·7
20–29	60/100	60		
			35–50	0·76
30–39	45·7/100	45·7		
			50–70	≃0
40–59	80/196	40·8		
60–70	80/197	40·6		

* All sera with titres of 8 and 16 were considered as negative, except for the 30–39 years and older age groups (see text).

† Calculated according to the formula set out in Materials and Methods.

‡ Postulated percentage.

persons acquiring infection, was used as a measure for the incidence of primary infections.

From the data in the third and fourth columns of Table 2 the annual infection risk can be estimated for successive age periods according to the formula set out in Materials and Methods. As indicated before, we consider the observed percentage of negative sera in any given age group as an estimate of susceptibility at the median age of this group. The highest risk (approximately 3 %) is found in adolescence and early adult life (last column of Table 2). After the age of 35 years the estimated risk is very low but the significance of this is questionable (see Discussion).

Risk of primary infection during pregnancy

To estimate the risk of primary infection during pregnancy two factors have to be taken into account, namely the age distribution of pregnant women in the population and the age-specific incidence of primary infections, i.e. the incidence in the total population of susceptible and immune persons. Data on the distribution of parturient women in The Netherlands in 1975 among five specified age periods are presented in the fourth column of Table 3. Precise quantitative data on the age distribution of parturient women are readily available, and were, therefore, used as substitutes for such data on pregnant women.

Estimates of incidences of primary infections for each of the age periods were

Table 3. *Calculated percentage of parturient women primarily infected during pregnancy*

Age (years)	Negative sera* (%)	Incidence of primary infections per 9 months (%)	Age distribution of parturient women, The Netherlands, 1975† (%)	Parturient women with primary infection during pregnancy (%)
17½	76·2			
		1·62	4·1‡	0·07
20	70·8			
		1·44	30·4	0·44
25	61·2			
		1·24	44·0	0·56
30	52·9			
		1·08	15·1	0·16
35	45·7			
		0·37	5·5§	0·02
45	40·8			
All ages			100·0	1·25

* Percentages for ages 17½ and 35 years were taken over from Table 2. Percentages for intermediate ages were interpolated for an annual infection risk of 2·02 %. Percentage for age 45 years was taken over from Table 2 (= percentage of 40–59 years group).

† Source: Centraal Bureau voor de Statistiek, Voorburg (The Netherlands).

‡ Inclusive < 17½ years.

§ Inclusive > 45 years (146 of 170249 parturient women of all ages).

obtained from the decrease in the percentages of negative sera within each period. The decrease between the median ages of 17½ and 35 years from 76·2 to 45·7 % (Table 2) is equivalent to an average annual infection risk (i.e. risk of non-immune persons becoming infected) of 2·02 % according to the formula set out in Materials and Methods. We used this figure to calculate the frequencies of negative sera at ages of 20, 25, and 30 years. This procedure to determine frequencies of negative sera is acceptable since the infection risk remained practically the same throughout this age period (Table 2). It was not applied beyond the age of 35 years because of the much lower estimated infection risk of persons over 35 years of age (Table 2). The calculated frequencies at the ages of 20, 25, and 30 years and the observed frequencies at the median ages of 17½, 35, and 45 years taken over from Table 2 are presented in the second column of Table 3. It should be noted that the percentages of negative sera of Table 2 were obtained from sera of both sexes. Differences per age group between frequencies for men and women were negligible, except in the 30–39 years age group (data not shown). As, within the full range of observations, this difference can be attributed to chance variation, the percentages are considered as valid for the female population.

The third column of Table 3 shows estimates of the incidences of primary infections per 9-month interval for each age period as calculated from the figures of the second column. For example, during the 30-month period between the ages of 17½ and 20 years the percentage of negative sera decreased by 5·4 %, that is, by 1·62 % ($\frac{9}{30} \times 5{\cdot}4$) per 9 months assuming a constant incidence within this age period.

https://doi.org/10.1017/S0022172400063191 Published online by Cambridge University Press

It is evident that, with a constant infection risk, the incidence of primary infections in a population of susceptible and immune persons actually decreases steadily with age because of the decreasing proportion of susceptible persons. Thus, the incidence decreased from 1·62 % per 9 months in the 17½–20 years age group to 1·08 % in the 30–35 years age group (third column of Table 3). The differences, however, between successive ages within each age group are small, and, for convenience, are neglected.

By applying the incidences of primary infections (third column of Table 3) to the corresponding age fractions of the population of parturient women (fourth column) an estimate of the percentage of primary infections for each age group of parturient women was obtained as shown in the last column. On the basis of these figures the incidence of primary infections in the total population of susceptible and immune pregnant women is estimated to be 1·25 % (total of the last column).

DISCUSSION

The age trend of seropositivity was interpreted as evidence of continuous risks of infection with Toxoplasma during the first decennia of life until the age of 50 years. The estimated annual infection risks were 0·5 % in early childhood, 2 % at school age, and 3 % during adolescence and early adulthood (Table 2, last column). These figures were obtained under the implicit assumption that all positive titres do reflect postnatal infections. As it is probable that in some infants, possibly 0·5 % (see below), antibodies have arisen as a result of an intra-uterine infection, the annual infection risks should be lowered slightly if they are to represent postnatal infections only. However, such a correction would mainly affect the estimated infection risk in early childhood and would leave unimpaired the obvious differences between young children and older persons.

For postnatal infections, two routes of transmission should be considered (Frenkel, 1973): consumption of raw meat and close association with infectious faeces of cats. In view of the high risk of infection in our study population during adolescence and in early adult life, it would seem that in this segment of the population the first route of transmission is by far the most important one. The popular out-of-doors consumption of snacks by adolescents and young adults might be a major factor. Extreme effects on seroconversion were observed by Desmonts *et al.* (1965) in a tuberculosis department of a hospital near Paris, where undercooked meat was served to children as a normal diet constituent. Of 641 children without antibodies at the time of admission 204 became seropositive during the course of the hospital stay. This corresponds with a *monthly* infection risk of 5·9 % as calculated according to the formula set out in Materials and Methods. High infection rates in childhood, which are attributed to the abundance of cats, suitable climatic conditions for sporulation of oocysts in soil, and playing habits of young children, were reported from four villages in northern Iran (Ghorbani, Edrission & Assad, 1978). The antibody positive rates rose from 17·3 % in the second year of life to 59·7 % for a 5–9 years age group.

The rather low frequency of positive sera in our study group upon entering the

age period of reproduction combined with an ensuing annual infection risk of 3 % results in a rather high percentage, namely 1·25, of parturient women with primary infection during pregnancy. The significance of this rate for the incidence of congenital toxoplasmosis, either clinical or subclinical, in newborns may be derived tentatively from observations by Desmonts & Couvreur (1974). These authors dealt with the outcome of 180 pregnancies – abortions excluded – complicated by primary maternal toxoplasmosis. Definite evidence of congenital infection was obtained in 59 cases, possible evidence in 11 cases. Thus, the risk of fetal infection was about 40 %. The ratio of subclinical to clinical infection of the child was 2:1. If it is assumed that the risk of fetal infection in maternal toxoplasmosis is the same in our population, we might expect five cases of congenital toxoplasmosis per 1000 births. In an earlier Dutch study (Koppe *et al.* 1974) in the obstetrical department of the Amsterdam university hospital 12 out of 1821 infants were found to be infected (6·6 per 1000).

Our estimates of the annual infection risks in different age groups were based on the assumption that the population at risk below 30 years of age includes all persons with negative (< 8) IF tests as well as those with titres of 8 and 16. That is, we assumed that these low titres represented false positive results. However, it cannot be excluded that the low titres may have been induced by cross-reacting antigens and, as cross-reacting antibodies, even have conferred some immunity to toxoplasma infection. Reactions due to cross-reacting antigens are usually lower in titre than specific reactions, and might be more transient. However, because antibodies to toxoplasma disappear slowly, it seems justified to accept – and this was in fact accepted – a part of the low titres in older persons as evidence of toxoplasma infections acquired in the more distant past. From this point of view negative sera (titre < 8) might even be considered as false negatives.

It could be argued that the finding of virtually constant titre distributions for the four 10-years age groups between 40 and 79 years reflects an equilibrium between conversions to seropositivity and reversions to seronegativity. This concept is supported by the finding of a rather high frequency, 8–9 %, of titres of 512 and higher, suggesting that the risk of infection continues above 40 years of age notwithstanding calculated annual infection risks near to zero (Table 2). On the other hand, it must be admitted that not all seronegatives are actually exposed to infection: absence of contact with infectious cat faeces and exclusion of undercooked meat in the food probably are an effective safeguard against toxoplasma infection. Therefore, seronegativity in elderly persons might be understood either as a consequence of reversion from earlier positivity or as an indicator of low degree or even absence of exposure in the past.

We wish to thank Mr T. F. H. Hermsen for his valuable technical assistance.

REFERENCES

DESMONTS, G. & COUVREUR, J. (1974). Congenital toxoplasmosis: a prospective study of 378 pregnancies. *New England Journal of Medicine* **290**, 1110–16.

DESMONTS, G., COUVREUR, J., ALISON, F., BAUDELOT, J., GERBEAUX, J. & LELONG, M. (1965). Etude épidémiologique sur la toxoplasmose: de l'influence de la cuisson des viandes de boucherie sur la fréquence de l'infection humaine. *Revue francaise d'Etudes cliniques et biologiques* **10**, 952–8.

FRENKEL, J. K. (1973). Toxoplasma in and around us. *BioScience* **23**, 343–52.

GHORBANI, M., EDRISSIAN, Gh. H. & ASSAD, N. (1978). Serological survey of toxoplasmosis in the northern part of Iran, using indirect fluorescent antibody technique. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **72**, 369–71.

KOPPE, J. G., KLOOSTERMAN, G. J., DE ROEVER-BONNET, H., ECKERT-STROINK, J. A., LOEWER-SIEGER, D. H. & DE BRUIJNE, J. I. (1974). Toxoplasmosis and pregnancy, with a long-term follow-up of the children. *European Journal of Obstetrics, Gynecology and Reproductive Biology* **4**, 101–10.

NUNEN, M. C. J. VAN & VEEN, J. VAN DER (1965). Examination for toxoplasmosis by the fluorescent antibody technique. *Tropical and Geographical Medicine* **17**, 246–53.

REMINGTON, J. S. & DESMONTS, G. (1976). Toxoplasmosis. In *Infectious diseases of the Fetus and Newborn Infant*, (ed. J. S. Remington and J. O. Klein), pp. 191–332. Philadelphia, London, Toronto: Saunders.