Epidemiol. Infect. (1992), **108**, 99–106
Printed in Great Britain

Longitudinal study of toxoplasma seroprevalence in South Yorkshire

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(*Accepted 29 August 1991*)

SUMMARY

Serum samples collected from individuals of a wide range of ages in South Yorkshire between 1969 and 1990 provided the basis for a longitudinal seroprevalence survey of *Toxoplasma gondii* antibodies. Sera numbering 3868 were screened for *T. gondii* specific antibodies using a commercial latex agglutination test. The resultant temporal series of serological profiles revealed a rise, with age, in seroprevalence, the rate of which showed a decrease through time. A plateau of around 40–50% prevalence was attained by the 41- to 45-year age-class in 1969 which was not approached until the 66- to 70-year class in the 1988–90 data set. This trend for decline in seroprevalence was confirmed by statistical analysis for the age range 21–60 years. These results may be indicative of a decrease in the rate of toxoplasma exposure in this study community over the 20-year period. The survey of 1988–90 provides a base-line profile of present-day seroprevalence in which 11% of individuals in the age range 16–45 years (roughly corresponding to the childbearing age-range) show evidence of past infection. The representative nature of the serum collection and public-health implications of these results are discussed.

INTRODUCTION

Infection in humans with the protozoan parasite *Toxoplasma gondii* in the United Kingdom is of interest for two main reasons. First, the parasite is the agent of congenital toxoplasmosis (CT), from which severe abnormalities can arise in the foetus and infant if infection is acquired by a mother during pregnancy [1–3]. Secondly, the parasite is of significance as an opportunistic infection in immunocompromised individuals such as heart-transplant and AIDS patients [4, 5].

Interest in toxoplasmosis and its public-health implications has been increasing in recent years. Much of the current debate centres upon the prevention of congenital infection and the merits of introducing a programme in the UK to monitor pregnant women for primary *T. gondii* infection [6–11]. Any assessment of the public-health significance of the infection will depend upon detailed epidemiological information with which to estimate accurately the magnitude of

the problem of human toxoplasmosis in the community and the present risk of infection in women of childbearing age [12].

Longitudinal clinical and serological studies and horizontal surveys of specific IgM to *T. gondii* can provide data on incidence of infection in women of childbearing age [13, 14], and surveillance programmes of infants and children have been used to assess numbers of cases of congenital infection [1, 2]. Horizontal age-stratified serological studies which measure the levels of specific antibody prevalence serve as a good basis for assessing the risk of infection in a community [15, 16]. In addition, longitudinal seroepidemiological studies can yield information of temporal change, the findings of which may subsequently aid the interpretation of horizontal surveys [17].

Little survey work on *T. gondii*-specific antibodies has been conducted since Fleck's horizontal cross-sectional study in 1969 [18]. Research since the publication of this survey has concentrated on the childbearing age band of the population and surveillance of cases of CT [1, 13]. In this present survey we have assessed the levels of *T. gondii* seroprevalence over a wide range of age-classes for a number of years dating from 1969 to 1990 in South Yorkshire, with the aim of improving our base-line knowledge on present levels of infection and of trends in rates of toxoplasma exposure through time.

METHODS

Serum samples

Serum samples numbering 3868, provided by the Public Health Laboratory Service at the Northern General Hospital in Sheffield, were collected over the period 1969–90, and stored, at −20 °C, in the Department of Experimental and Clinical Microbiology at Sheffield University Medical School. The sera were originally obtained for routine diagnostic purposes from individuals in the South Yorkshire region (Sheffield, Rotherham, Doncaster and Barnsley conurbation), and over the period of collection were mostly from patients with, or suspected of having, pneumonia, rubella, pyrexia of unknown origin (PUO) or influenza. In the past, sera were also obtained from probable cases of hepatitis, and a small proportion of sera over the period of collection were from patients suspected of suffering from toxoplasmosis. The sera were obtained from blood specimens arriving at the PHL during the months of June and July each year, and the collection has been specifically undertaken to provide a yearly bank of sera from all age groups for influenza seroepidemiology studies.

As shown in Table 1, the sample collection consists of a series of independent (i.e. comprising unrelated individuals) cross-sectional samples, each separated by a period of approximately 3–4 years and each spanning a wide range of age-classes (0–80+ years). Approximately equal numbers of samples were collected in a single year. For the purposes of our analyses years 1988–90 have been pooled and treated as a single horizontal sample set.

Serological screening

Sera were screened at Imperial College, London, using an indirect latex agglutination (LA) test (Toxoreagent-MT 'Eiken', Eiken Chemical Company,

Table 1. Toxoplasma gondii *antibody prevalence* (%) *stratified by 5-year age-classes from surveys in South Yorkshire between 1969 and 1988–90* (r = *number seropositive and* n = *sample size*)

	Date of survey													
	1969		1973		1976		1979		1981		1985		1988–90	
Age (years)	*r/n*	%	*r/n*	%	*r/n*	%	*r/n*	%	*r/n*	%	*r/n*	%	*r/n*	%
0–5	0/43	0·0	2/29	6·9	0/25	0·0	1/27	3·7	1/15	6·7	0/27	0·0	3/92	3·3
6–10	1/18	5·6	0/18	0·0	0/7	0·0	0/21	0·0	0/21	0·0	2/30	6·7	6/81	7·4
11–15	0/8	0·0	1/4	25·0	8/24	33·3	2/25	8·0	4/23	17·4	1/21	4·8	8/76	10·5
16–20	6/49	12·2	2/50	4·0	3/25	12·0	3/27	11·1	6/32	18·8	1/25	4·0	10/94	10·6
21–25	15/76	19·7	5/28	17·9	2/24	8·3	2/24	8·3	3/24	12·5	4/26	15·4	6/93	6·5
26–30	6/19	31·6	2/17	11·8	5/24	20·8	2/21	9·5	3/21	14·3	4/22	18·2	8/77	10·4
31–35	11/32	34·4	6/18	33·3	6/25	24·0	7/23	30·4	5/26	19·2	4/27	14·8	5/87	5·7
36–40	8/28	28·6	5/24	20·8	6/26	23·1	6/24	25·0	2/21	9·5	3/25	12·0	10/80	12·5
41–45	13/26	50·0	10/26	38·5	10/25	40·0	6/29	20·7	1/25	4·0	5/21	23·8	15/79	19·0
46–50	9/14	64·3	4/19	21·1	6/24	25·0	5/22	22·7	7/28	25·0	7/27	25·9	14/76	18·4
51–55	3/9	33·3	9/21	42·9	6/24	25·0	8/22	36·4	3/14	21·4	14/30	46·7	20/82	24·4
56–60	7/14	50·0	15/27	55·6	4/9	44·4	10/27	37·0	15/32	46·9	6/21	28·6	27/75	36·0
61–65	8/15	53·3	15/29	51·7	6/14	42·9	10/18	55·6	9/21	42·9	12/27	44·4	22/74	29·7
66–70	7/19	36·8	10/21	47·6	10/28	35·7	11/30	36·7	12/23	52·2	10/20	50·0	30/73	41·1
71–75	7/12	58·3	13/27	48·1	13/27	48·1	12/21	57·1	11/25	44·0	12/26	46·2	36/82	43·9
76–80	7/12	58·3	9/24	37·5	14/21	66·7	12/22	54·5	10/21	47·6	13/26	50·0	42/88	47·8
80+	6/10	60·0	20/37	54·1	16/30	53·3	11/17	64·7	5/15	33·3	18/45	40·0	60/121	49·6
Sample size	404		419		382		400		387		446		1430	

Japan; available from Mast Diagnostics Ltd, Derby Road, Bootle, Merseyside L20 1EA), the principle of which is the formation of patterns of agglutination of latex particles coated in inactivated toxoplasma antigen in the presence of specific antibodies. Published studies have shown this LA test to compare favourably with the Sabin–Feldman Dye Test (considered the reference serological technique) for screening purposes [19, 20]. A single batch of kits was used throughout the survey to minimize test-induced variability.

Test procedure followed manufacturer's instructions except for a modification to assess presence or absence of specific antibodies rather than a specific titre of antibody in a serum sample. For this purpose only two dilutions of each sample, 1:16 and 1:32, were set up. Positive latex agglutination (i.e. an agglutination pattern of 1 or more using manufacturer's guidelines) at the 1:16 dilution was taken as indicative of seropositivity, and the 1:32 dilution served as a safeguard against false negative readings due to prozone at 1:16.

Statistical methods

Statistical analyses for each cross-sectional sample were carried out on data grouped into 10-year age-classes which increased sample sizes. Differences in seroprevalence between sexes for each survey year with k age-classes were assessed using a $2 \times k$ chi-square test in which expected numbers seropositive were estimated assuming independence between presence of specific antibodies and sex, but not age. Time-dependent changes in seroprevalence were analysed by age-class using a chi-square test which is particularly sensitive to trend in proportions [21]. The calculated trend statistic, χ_1^2, suggests a significant regression in seroprevalence through time if it exceeds the 5% probability level ($P < 0{\cdot}05$, 1 degree of freedom). A residual chi-square, χ_2^2, calculated from the difference between the overall chi-square, χ^2 (assuming total independence between variables), and the trend chi-square, tests for departure from linear regression resulting, for example, from some higher-order regression function [21]. Residual chi-square values which do not exceed the 5% probability level (i.e. $P > 0{\cdot}05$, $k-2$ degrees of freedom) indicate no departure from a linear trend through time.

RESULTS

Table 1 records the number of sera positive by the LA test for toxoplasma-specific antibody, total numbers of samples tested and the proportion seropositive, by age-class, for each cross-sectional serum collection. Assuming the presence of antibody to be indicative of *T. gondii* infection sometime in the past and that some classes of *T. gondii*-specific antibodies are of life-long duration, these data record the changes through age and time in the proportion of the study population who have experienced toxoplasma infection. Statistical comparison of proportions seropositive by sex of individual revealed no significant differences. Further analysis was therefore confined to data pooled from both sexes.

Using data recorded in Table 1, three-point moving averages for proportions seropositive were calculated and are presented as a 3-D surface in the figure. The graph clearly reveals, for each cross-sectional profile, a rise with age in the proportion seropositive (i.e. who have experienced infection), to a maximum of

Table 2. *Analysis of time-dependent trends in toxoplasma seroprevalence in South Yorkshire*

Age class (years)	χ_1^2 (D.F. = 1)	P	χ_2^2 (D.F. = 7)	P
1–10	2·27	N.S.	1·66	
11–20	0·22	N.S.	12·89	
21–30	8·26	< 0·01	3·14	N.S.
31–40	19·95	< 0·001	1·98	N.S.
41–50	14·79	< 0·001	10·90	N.S.
51–60	5·04	< 0·025	3·59	N.S.
61–70	1·86	N.S.	2·91	
71–80	0·49	N.S.	3·19	
80+	0·99	N.S.	4·70	

Where N.S. is not significant, D.F. is degrees of freedom, χ_1^2 is chi-square trend statistic, and χ_2^2 is residual chi-square = $\chi^2 - \chi_1^2$.
See Statistical Methods and [21] for more detail.

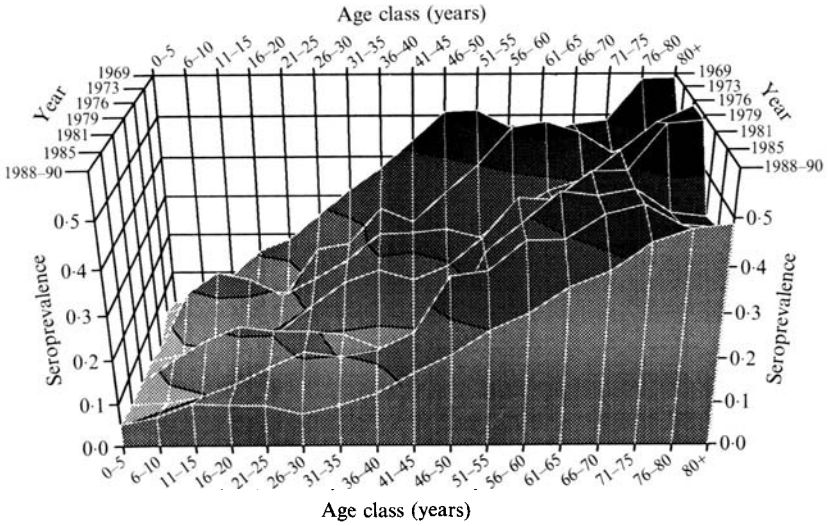

Fig. 1. Age-related changes in toxoplasma seroprevalence for a number of cross-sectional surveys between 1969 and 1988–90 in South Yorkshire (sample sizes are given in Table 1). Data have been smoothed using three-point moving averages (see text for details). Boundaries between shades of grey represent isoclines of equal seroprevalence.

around 50% in the adult age-classes. The rise with age in proportions positive appears to be most rapid in the 1969 survey, declining progressively through time to the 1988–90 survey. Note from the graph that whereas a level of 50% seroprevalence is attained by age-class 41–45 years in 1969, in more recent survey years (1985 and 1988–90) such a level is not approached until the 66- to 70-year class or more. The trend for decline is highlighted by the isoclines of seroprevalence in the figure, which tend to migrate to the older age-classes through time. This observation is further endorsed by statistical analysis of trend in seroprevalence through time by age-class, which revealed significant linear regression for the age-classes 21–30 to 51–60 years as shown in Table 2.

Present-day seroprevalence, as indicated by results from the 1988–90 survey

https://doi.org/10.1017/S0950268800049542 Published online by Cambridge University Press

(Table 1), in the age-range 16–45 years (which roughly corresponds to the childbearing age-classes) has a mean value of 10·8%.

DISCUSSION

Serum samples collected from individuals of a wide range of ages over the period 1969–90 have been used to provide base-line data on present-day toxoplasma seroprevalence in a study population of South Yorkshire and insights into the time-dependent epidemiology of *T. gondii* infection in this community. Samples were originally taken for routine diagnostic purposes and were screened for toxoplasma-specific antibodies using a well-evaluated latex agglutination test that is known to produce similar results to the standard dye-test. Results pooled from the years 1988–90 provide current day estimates of seroprevalence which indicate that roughly 10% of individuals under the age of 40 years have experienced infection, rising slowly to around 50% in those of 75 years or over. Approximately 11% of individuals within an age-range roughly spanning the childbearing age-classes (16–40) had evidence of remote *T. gondii* infection. Comparison of horizontal age-serological profiles from serum sets collected between 1969 and 1988–90 reveals temporal trends in the pattern of toxoplasma seroprevalence over the past 20–22 years.

Interpretation of these results in relation to toxoplasma infection rates in the study community requires closer consideration of the nature of the serum sample sets and demographic patterns in the area of South Yorkshire. With regard to the collection of serum samples, the issues of concern are, first, to what degree these samples are representative of the catchment population of South Yorkshire (important to the interpretation of the magnitude of seroprevalence estimates), and second, whether or not this representativeness (in whatever degree) has altered over the duration of the study (important to the interpretation of trends in seroprevalence). Sera stored by the Northern General Hospital Public Health Laboratory (PHL) were originally taken for the investigation of a range of disorders potentially of microbial origin. This alone does not constitute a bias in sampling so far as the taking of a blood sample for laboratory investigation is not indicative of a person of below average health. The PHL material included a small proportion of samples collected from patients suspected of suffering from toxoplasma infection. The extent to which this might result in overestimation of seroprevalence is difficult to assess since the exact number and age distribution of individuals referred for this reason is unknown. However, the proportion of such referrals is known to be small and suspected to be of the same magnitude as for similar laboratories. There has been no major change in emphasis of referral of samples to the PHL at the Northern General Hospital over the study period. The subset of samples intended for influenza seroepidemiological studies were selected at the same time each collection year in roughly equal numbers in total and in each age-class. No other selection criteria were used and the collection protocol has not varied since initiated. Collected sera were stored at −20 °C and screened using a single batch of the commercial LA test.

The above statements suggest that the method of collection of samples comprising this study was unlikely to impart major bias on the results with respect

to temporal trends in seroprevalence in the study community. The non-exclusion of sera from suspected toxoplasma patients is of potential importance to the representativeness of seroprevalence estimates of the study community, although we suspect that such error is likely to be small. In the light of these facts we suggest that the observed progressive decline in seroprevalence over the study period may have two possible explanations. The first is demographic; that of migration into or out of the study area. The results would, however, only be consistent with a more or less steady imbalance towards the emigration of seropositives or influx of individuals from areas of lower seroprevalence over the last two decades. A second, and we would suggest, more plausible explanation, is that as a result of changes in the rate of exposure of susceptibles to tissue cysts or oocyst-contaminated environment, toxoplasma infection rates have declined in the study area since 1969. No information was available on the socio-economic status or nature of employment of individuals from whom the sera were acquired which may have provided insights as to the origin of factors which could be responsible for reduced rates of exposure.

The degree to which the results yielded from this study reflect countrywide patterns in the general population is unknown. It is notable that the results of our 1969 serum set accord well with those of Fleck [18], whose survey in 1969, using the dye test, comprised a wide age-range of healthy individuals from England and Wales [22]. However, recent studies of HIV seropositive individuals [23] and antenatal mothers [13] yielded higher seroprevalence levels than those of the same age in the 1988–90 sample set of this study. Clearly there is a need to conduct further studies to ascertain seroprevalence levels in the general population from other regions. Countrywide changes in *T. gondii* seroprevalence similar to those observed in this study over the last 20 years could have important public-health implications. Declining levels of *T. gondii* seroprevalence due to declining rates of exposure would be of significance in assessing the likelihood of reactivation of toxoplasma tissue cysts in immunocompromised patients. Furthermore, a temporal decline in the rate of toxoplasma infection would have a critical bearing on the estimation of rates of infection in women of childbearing age from present-day horizontal cross-sectional serological data. Significant error in the estimation of risk of exposure and, subsequently, of congenital toxoplasmosis, may arise if interpretation does not take account of age-related *and* time-dependent changes.

ACKNOWLEDGEMENTS

We gratefully acknowledge the support of the Medical Research Council (JW, DJN) and The Royal Society (DJN). We thank the Public Health Laboratory at the Northern General Hospital for allowing us access to their serum samples.

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