Community study of toxoplasma antibodies in urban and rural schoolchildren aged 4 to 18 years

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Abstract

To estimate the prevalence of toxoplasma antibodies in schoolchildren and their association with clinical and environmental data, antibody titres were measured in 1276 children aged 4 to 18 years attending primary and secondary schools. Environmental and clinical data were obtained by questionnaire. Altogether 12.8% (163/1276) of children had antibodies to *Toxoplasma gondii* with no difference between the sexes. Seroprevalence was higher in country children (16.6% (50/302)) than town children (10.2% (75/737)). The proportion testing positive increased with age in both town and country children. No association with cat ownership was found. Toxoplasma seropositivity was associated with a positive toxocara titre, having had a bitch whelp in the past two years, and having an unwormed dog at home. Lack of energy or tiredness in the last 12 months were the only clinical features associated with a positive titre.

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Toxoplasmosis is an important infection of humans. The natural host of *Toxoplasma gondii* is the cat. Congenital infection of the human fetus may cause abortion, blindness, mental retardation, and other neurological disease while acquired infection is usually more benign in the immunocompetent subject.

While the seroprevalence of toxoplasmosis in adults, especially pregnant women, has been recorded in many studies, we have found no community based study of toxoplasma seroprevalence in schoolchildren in the British Isles and only one in Europe.[1] We record here the seroprevalence of *T gondii* infection in 1276 schoolchildren.

Subjects and methods

STUDY POPULATION

The investigation formed a sequel to a report of *Toxocara canis* infection in schoolchildren in which details of the study population are given.[2] In that study, 30 schools were randomly selected from the Department of Education's list of schools for the county of Dublin and the adjoining counties of Wicklow and Kildare. Fifteen schools agreed to take part. Consent forms and questionnaires were sent to parents for completion. Questionnaires were returned for 2818 children and 2134 of these provided venepuncture blood samples from which haematology results were obtained. Bloodspots from the venepuncture specimens were taken onto Guthrie cards from 1276 children's samples in seven schools. There were 652 girls and 624 were boys, which is similar to the sex distribution of the 1991 national census for this age range. The ages of those providing Guthrie card samples was from 4 to 18 years. Ages are given as completed years of age so that '6 years' denotes a child ≥ 6 and <7 years of age. Children were designated as having an urban or rural residence depending on the location of the school as children attend their local primary school. Written parental consent had been obtained for venepuncture but blood was taken only if the child consented at the time of phlebotomy. Parents of some smaller children were present when blood was taken. The study was approved by the ethics committee of the Federated Dublin Voluntary Hospitals and St James's Hospital.

TOXOPLASMA SEROLOGY

The modified latex agglutination test for *T gondii* antibody (Eiken Toxoreagent) was used to screen the Guthrie card samples. Equivalent serum titres are 4.13 times higher than the results obtained using this method.[3] A titre of $\geq 1{:}4$ is regarded as positive. Toxocara titres were measured as previously reported.[2]

QUESTIONNAIRE DATA

Questionnaire data were available from the toxocara study questionnaire and the replies provided data for lifetime prevalence of asthma, eczema, hay fever, earth eating, thumb or finger sucking, convulsion; the presence, in the past two years of cats, dogs, birds, fish, and the birth of puppies in and around the home; the 12 month prevalence of abdominal pain, nausea, vomiting, headache, cough, wheeze, poor appetite, lack of energy or tiredness, behaviour upset, fever, and skin rash. Those with a dog at home were asked whether it was wormed once a year (or more often). The χ^2 test was used for statistical analysis.

Results

Altogether 12.8% (163/1276) of samples tested positive. There was no difference between the sexes: girls 12.7% (83/652), boys 12.8% (80/624). The prevalence of positive results increased significantly with increasing age (χ^2 25.82, df 11, $p = 0.0069$ after combining age groups 4 and 5 and 16, 17, and 18 to provide adequate numbers; table 1).

Altogether 87.2% (1113/1276) of the study population had negative titres, 1.6% (20) had titres $\geq 1{:}4$ and $<1{:}16$, 1.9% (24) had titres

Table 1 Number and percentage of schoolchildren testing positive for T gondii antibodies in each age group

Age group (years)	All children		Country		Town	
	No	*No (%) positive*	*No*	*No (%) positive*	*No*	*No (%) positive*
4+5	96	6 (6.3)	40	3 (7.5)	56	3 (5.4)
6	103	7 (6.8)	37	3 (8.1)	66	4 (6.1)
7	126	18 (14.3)	51	7 (13.7)	75	11 (14.7)
8	122	16 (13.1)	33	8 (24.2)	89	8 (9)
9	122	12 (9.8)	37	4 (10.8)	85	8 (9.4)
10	149	17 (11.4)	38	3 (7.9)	111	14 (12.6)
11	167	26 (15.6)	37	13 (35.1)	130	13 (10)
12	132	23 (17.4)	26	9 (34.6)	106	14 (13.2)
13	73	3 (4.1)	3	0	70	3 (4.3)
14	69	16 (23.2)			69	16 (23.2)
15	65	9 (13.8)			65	9 (13.8)
16–18	52	10 (19.2)			52	10 (19.2)

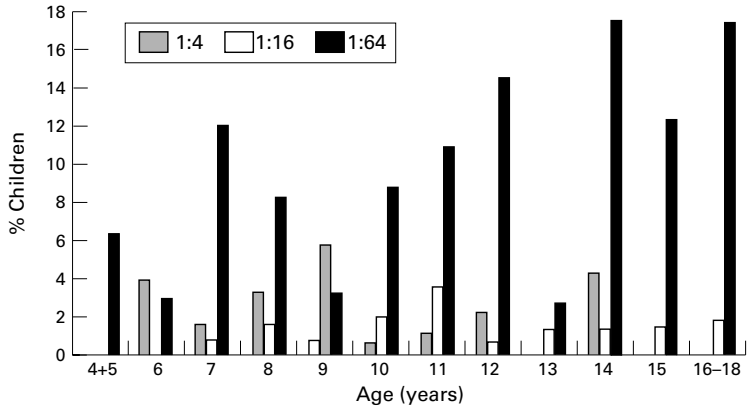

Figure 1 The percentage of children with antibody titres to T gondii in each age group. Titres of ⩾1:4 and <1:16 are recorded as 1:4, those ⩾1:16 and <1:64 as 1:16, and those ⩾1:64 as 1:64. Equivalent serum titres are 4.13 times higher than those from the Guthrie cards, so that a titre of ⩾1:64 is equivalent to a serum titre of ⩾1:264.

⩾1:16 and <1:64, and 9.3% (119) had titres ⩾1:64 There was an overall rise in the proportion of children with titres of ⩾1:64 (equivalent to a serum titre of ⩾1:264) with increasing age (fig 1).

Country children were more commonly seropositive than town children (table 1). In the population examined the oldest country child was 13.35 years, so the town/country comparison was made for children ⩽13.35 years. Altogether 16.6% (50/302) country children were positive while the figure for town children was 10.2% (75/737) (χ^2 8.239, df 1, p = 0.0041, difference = 6.4%, 95% confidence interval (CI) 1.7% to 11.1%).

Positive results were no more common in those with a cat, dog, bird, or fish in or around the house in the past two years. There was no association between seropositivity and having a bitch whelp at any time. However 32.5% of those who were seropositive had a bitch whelp in the past two years while the figure was 16.9% for those who were seronegative (χ^2 5.526, df 1, p = 0.0187, difference = 16.6%, 95% CI 0.4% to 30.8%). There was a significant association between having a positive toxocara titre (⩾1:50) and having a positive toxoplasma titre (χ^2 35.74, df 1, p ⩽ 0.0001). Of the toxoplasma positive children 51.9% (82/158) were toxocara positive while 28.3% (304/1075) of the toxoplasma negative children were toxocara positive (difference =23.6%, 95% CI 15.4% to 31.9%).

There was an association between not worming dogs once a year or more often and a positive toxoplasma titre. Of those who wormed their dogs 11.3% (50/444) tested positive while 19.2% (29/151) who did not were positive (χ^2 6.176, df 1, p=0.013 difference = 7.9%, 95% CI 1.0% to 14.8%).

Toxoplasma infection does not cause eosinophilia, but more seropositive (45.3%) than seronegative children (35.8%) had an eosinophil count of $\geqslant 0.4 \times 10^9$/l ($\chi^2$ 5.458, df 1, p = 0.0195, difference = 9.5%, 95% CI 1.3% to 17.7%). However this association was not significant at higher cut off levels for the eosinophil count, which are more appropriate values for eosinophilia.[4]

Of the clinical features examined by questionnaire only the answer to one question was associated with a positive titre (In the last 12 months has your child had any of the following?—lack of energy or tiredness.) Of the seronegative children 14.5% (158/1093) complained of lack of energy or tiredness while 21.3% (34/160) of the seropositive children did so (χ^2 4.966, df 1, p = 0.0259, difference = 6.8%, 95% CI = 0.12% to 13.5%). There was no significant difference in the proportion reporting lack of energy or tiredness between those with titres ⩾1:4 and <1:64 and those with titres ⩾1:64 but the numbers in the first group were small (fig 1). There was no association with earth eating or digit sucking.

Discussion

The major application of testing Guthrie card samples is to study congenital disease; however the samples may also be used for other applications. The modified latex agglutination test for *T gondii* antibodies when used on Guthrie card eluates detected 98.8% of positive serum samples, with 100% specificity when compared to the standard latex agglutination test. The two eluates in which antibody was not detected came from patients with equivocal results by the standard test.[3] The standard Eiken latex test has been found to have a sensitivity of 99% and a specificity of 81%.[5] Other methods for detecting toxoplasma antibodies (such as the IgM immunosorbent agglutination assay) can detect low levels of specific antibody which may not be detected by the toxoplasma dye test or latex agglutination test.[6] In the same study comparison of dye test results obtained from filter paper eluates and from serum samples showed that the lowest level of toxoplasma specific IgG detected from filter paper eluates was 10 IU. This lower limit may also apply to the latex agglutination test applied to Guthrie card samples in which case antibody levels below 10 IU will have been falsely recorded as negative and so the recorded seropositivity rate will be lower than that which would have been found using blood samples and the toxoplasma dye test. While Guthrie card samples can be tested for specific IgM this estimation was not undertaken in the present study because of the cost involved.

We have found no reports of community based studies of toxoplasma antibody levels in

Table 2 Seroprevalence rate, as a percentage, reported in a hospital based South Yorkshire[7] series and in the present study. The absolute values are given in brackets. Both studies used the Eiken Toxoreagent latex agglutination test for analysis. The children in the present study were aged 4 to 18 years

Age (years)	South Yorkshire 1988–90 (hospital study)	Ireland 1992–3 (community study)
0–5	3.3 (3/92)	6.3 (6/96)
6–10	7.4 (4/81)	11.3 (70/622)
11–15	10.5 (8/76)	15.2 (77/506)
16–20	10.6 (10/94)	19.2 (10/52)

schoolchildren in the British Isles and only one in Europe.[1] Huldt *et al* in a community based study of 15 to 16 year old Scandinavian children found a seroprevalence of 19% in girls and 14% in boys.[1] An exact seroprevalence rate for other age groups was not recorded. In contrast to the present study they reported that antibodies were more common in girls than boys at all ages. Walker *et al* reported data from small numbers of hospital based South Yorkshire children gathered into five year age groups over the years 1988–90.[7] They used the Eiken Toxoreagent latex agglutination test for analysis. All of their rates were lower than those of the equivalent age group in the present study (table 2). Etheredge and Frenkel examined 760 Panamanian children aged 0 to 12 years from 13 localities and reported values from 0% to 45%.[8] They failed to find an association with age or sex. The zero prevalence rates were from three islands where toxoplasma infection was not found in the local cats. Huldt *et al* found no association between antibody titres and the presence of cats in the family or the consumption of raw meat by family members.[1]

The reason for the rise in quantitative titres (fig 1) with age is not clear. An hypothesis would be that the increase is a reflection of increasing 'exposure years' as the children get older. Multiple minor infections might at first produce low antibody levels and later higher levels.

Frenkel *et al*, in a study of toxoplasma transmission to children, reported a relative risk for contact with nursing dogs which was higher than the relative risk for cats.[9] It is of interest that this association between nursing dogs and toxoplasma antibodies should be found both in Panama and in Europe. In the present study no association was found with any of the pets or working dogs in or around the home, other than for having had a bitch whelp in the past two years and for having unwormed dogs. Frenkel *et al* suggested that dogs might be instrumental in mechanical transmission by eating or rolling in cat faeces,[9] but this hypothesis seems tenuous as in their data dog contact alone was associated with a seropositivity rate of 4.2% and no contact with dog or cat was associated with a rate of 14.4%. However, the association with dog contact is further supported by the association between toxoplasma and toxocara seropositivity. It may be that this association and the association with unwormed dogs indicate an alternative route of infection involving dogs or are merely markers for suboptimal animal husbandry and lax hygiene where animals are concerned. The lack of association with cat ownership suggests that either most infection is acquired through the food chain or other routes of ingestion or that environmental contamination by cats is so widespread that owning a cat has little effect on the level of exposure to toxoplasma infection. No information on the children's feeding habits was available from the questionnaire.

With the exception of the 10 year age group, in all age groups up to 12 years a higher proportion of country children than town children had titres $\geq$1:64 (table 1). By the age of 13 years the cumulative percentage of country children with titres $\geq$1:64 was 12.4% (37/299) while that for town children was 7.1% (51/719). These findings are in keeping with those of French *et al* who showed (in adults) a negative association with animals with a 9.4% seroprevalence in stock handlers with intimate animal contact but little land contact and a 34.9% seroprevalence rate in adults working the land.[10] Beattie also reported a higher prevalence of dye test positivity in adult country dwellers than town dwellers.[11]

The finding of lack of energy or tiredness in 21.3% of seropositive children is in keeping with the report of Beverley and Beattie that fatigue was a common and, in some cases, a long continued feature.[12] Their study group of 30 subjects included both adults and children. In the present study the questionnaire only inquired about the previous 12 months regarding this symptom.

Using the data it is possible to extrapolate beyond the data envelope to obtain an estimate of the congenital infection rate. This extrapolation extends two years below the youngest group of subjects and involves data which show considerable year to year variation. Any estimate is likely to be crude. If the seropositivity rate is extrapolated using all the data an estimate for 1 year olds is 2.2% seropositive and for those 6 months old 1.6% ($y = 1.181x + 1.016$. R squared for this regression = 43.4% (y = the percentage of children seropositive and x = the age in years)). If the country children are excluded in an attempt to reduce variation in the data these figures become 0.9% and 0.3% ($y = 1.222x - 0.336$; R^2 for this regression = 44.7%). We do not regard these figures as providing anything more than an indication that the congenital toxoplasmosis rate may be low. Taking logarithms and applying non-linear regression models do not help the fit of the curve.

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