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## Risk factors for toxoplasmosis in pregnant women in Kent, United Kingdom

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### SUMMARY

The aim of this study was to establish the relative importance of various risk factors for toxoplasmosis in a United Kingdom antenatal population. Toxoplasma immune status was determined by an immunoassay and linked to a questionnaire exploring dietary and environmental exposure to toxoplasmosis. The overall seroprevalence found was 9·1% (172/1897). A significantly higher seroprevalence was associated with rural location of the childhood home, childhood home in Europe excluding the United Kingdom, feeding a dog raw meat and increased age. A non-significant higher prevalence of toxoplasmosis was observed in women who had lived with a cat or kitten as a child. In contrast to recent European studies only weak associations between diet and toxoplasmosis were found. Gardening activity was not associated with seropositivity but a non-significant lower seroprevalence was seen in gardeners who always wore gloves. This study confirms that toxoplasma prevalence in the United Kingdom has continued to decline since the 1960s. The increasing seroprevalence with age found in this study, highlights the continuing need to educate women of childbearing age about the risk factors for toxoplasmosis.

### INTRODUCTION

Toxoplasmosis is usually a self-limiting infection in immunocompetent adults but can cause congenital infection with significant fetal morbidity and mortality when primary infection occurs during pregnancy [1–3]. Chorioretinitis with significant visual loss can also be a late complication of congenital infection [4, 5] in children who were asymptomatic at birth. Infection is acquired by ingesting oocysts excreted in cat faeces or eating raw or undercooked

meat from infected animals containing tissue cysts. The relative importance of these two sources of infection in the United Kingdom is unknown and provides the purpose for undertaking this study.

In contrast to France and some other European countries where toxoplasmosis is more common, UK antenatal clinics do not screen for toxoplasmosis in pregnancy. However, pregnant women are issued with advice about avoiding dietary and environmental exposure to toxoplasmosis. The need for more information about toxoplasma epidemiology in the United Kingdom was identified in a 1990 PHLS working party report on TORCH screening [6] and was reiterated in a Royal College of Obstetrics and Gynaecology multidisciplinary report of 1991.

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Recent studies involving pregnant women have confirmed the relatively low UK prevalence of toxoplasmosis [7–9] and a significant association between seropositivity for toxoplasmosis and country of birth and ethnic group [8]. However, dietary and environmental sources of toxoplasmosis have not been examined in any recent studies involving the UK population.

## METHODS

Pregnant women attending antenatal clinics in the Ashford, Folkestone and Dover areas of East Kent between October 1999 and November 2001 were invited to join this study which involved testing ‘booking’ blood samples for toxoplasma antibodies and completing a questionnaire about dietary and environmental exposure to toxoplasmosis. Dietary questions were phrased to elicit information about habits immediately prior to discovery of pregnancy. All women were counselled about the implications of finding evidence of toxoplasma infection including the small risk that amniotic fluid sampling might be recommended if blood samples indicated recent infection. All blood samples were screened for toxoplasma IgG antibodies using the Abbott AxSYM (Abbott Laboratories, Abbott Park, IL, USA) immunoassay system. Samples positive for toxoplasma IgG antibody were also screened for toxoplasma IgM antibody as an indicator of recent infection. All IgM antibody-positive samples were referred to the PHLS Toxoplasma Reference Laboratory for further studies to establish the timing of infection in relation to the pregnancy. When appropriate, amniotic fluid samples were examined by polymerase chain reaction (PCR) [10] for evidence of active infection. The questionnaires were completed by all consenting women before toxoplasma antibody results were available, thus minimizing any bias that might result from personal knowledge of antibody status. Questionnaire results were scanned into an Access database using Formic software and linked to matching laboratory results. A multivariable statistical analysis of all dietary and environmental risk factors was carried out.

### Statistical method

Single variable analysis was carried out in Epi-Info (Epi 6.04 d, Centers for Disease Control and Prevention, Atlanta, GA, USA). We compared women seropositive for toxoplasmosis to seronegative women.

The  $\chi^2$  or Fisher’s exact test were used to see if there were any significant differences between the cases and controls in their exposure to hypothetical risk factors for acquisition of toxoplasmosis. Where appropriate the  $\chi^2$  test for trend was used to compare quantitative or ranked exposure. Variables which had  $P < 0.2$  and were not protective were then put into a multivariable logistic regression model. A backwards stepwise procedure was undertaken with age being forced into the model, although it was not significant. All the two-way interactions of the variables in the final model were tested. Multivariable analysis was carried out using GLIM software [11]. Population-attributable fractions for significant risk factors identified using logistic regression were calculated using the Aflogit command of STATA version 6.0 [12].

## RESULTS

The number of women recruited to the study was 1923. Two women were eliminated because insufficient serum samples were received in the laboratory. Inadequately completed or wrongly labelled questionnaires were received from 26 women leaving a total of 1897 women from whom adequate questionnaires linked to toxoplasma antibody results were available for analysis. The overall seroprevalence of toxoplasmosis in the study population was 9.1% (172/1897). Twelve women were positive for toxoplasma IgM antibodies of whom two were judged to be at risk of congenital infection following further analysis by the Toxoplasma Reference Laboratory. One of the two women underwent amniotic fluid sampling and had negative results for toxoplasma DNA using a PCR assay. The other woman with evidence of recent toxoplasma infection underwent a termination of pregnancy for reasons unconnected with her toxoplasma antibody status.

### Statistical analysis

Tables 1 and 2 summarize the results of the  $\chi^2$  single variable analysis of qualitative exposure to risk factors for toxoplasmosis.

The  $\chi^2$  test for trend was used for frequency of gardening activity, use of gloves when handling soil, length of time at current home, frequency of handling raw meat in the kitchen, and frequency of eating various types of takeaway food. None of these were found to have  $P$  values of  $< 0.2$  (results not shown). The  $\chi^2$  test for trend was also used for frequency of

Table 1. Single variable analysis of life-time risk factors for toxoplasma seropositivity in pregnant women, ascertained by questionnaire completed without knowledge of immune status

Potential risk factor		Cases seropositive	Controls seronegative	RR	95% CI	P value
Cat living in house as adult	No	66	723			0.393
	Yes	106	996	1.15	0.86–1.54	
Kitten living in house as adult	No	91	946			0.621
	Yes	81	768	1.09	1.03–2.34	
Cat living in house as child	No	49	745			<0.001
	Yes	122	976	1.80	1.31–2.48	
Kitten living in house as child	No	64	852			0.002
	Yes	108	860	1.60	1.19–2.15	
Responsible for feeding cat/kitten	No	59	665			0.327
	Yes	112	1056	1.18	0.87–1.59	
Often fed cat/kitten raw meat	No	143	1540			0.021
	Yes	28	177	1.61	1.10–2.35	
Ever cleaned or emptied cat litter tray	No	75	771			0.856
	Yes	96	946	1.04	0.78–1.39	
Dog living in home as adult	No	69	752			0.432
	Yes	102	965	1.14	0.85–1.52	
Dog living in home as child	No	48	610			0.062
	Yes	123	1108	1.37	0.99–1.89	
Often fed dog raw meat	No	138	1528			0.001
	Yes	34	189	1.84	1.30–2.61	
Description of garden	No garden	18	185			0.650
	Grass only	37	359	1.06	0.59–1.91	
	Flowerbeds	98	1015	0.99	0.59–0.68	
	Veg. plot	0	7	0.04	<0.001–58.4	
	Veg. & flowerbeds	19	147	1.33	0.67–2.62	
Smoking habit	Non smoker	79	827			0.607
	Smoker	93	885	1.09	0.82–1.45	
Smoking while handling raw meat	No	167	1683			0.188
	Yes	5	25	1.85	0.82–4.16	
Smoking while gardening	No	150	1536			0.359
	Yes	21	166	1.26	0.82–1.94	
Location of childhood home	Within 50 miles	101	1191			<0.001
	More than 50 miles	38	382	1.17	0.79–1.73	
	Europe, excl. UK	20	48	4.91	2.81–8.60	
	Overseas	11	59	2.20	1.12–4.32	
Description of childhood home	Town garden	69	946			<0.001
	Town, no garden	8	58	1.89	0.87–4.12	
	Village	46	542	1.16	0.79–1.71	
	Countryside	29	114	3.49	2.17–5.61	
	Farm	15	47	4.38	2.33–8.22	
Description of current home	Town garden	85	905			0.013
	Town, no garden	11	159	0.74	0.38–1.41	
	Village	47	509	0.98	0.68–1.43	
	Countryside	19	93	2.18	1.27–3.74	
Eaten beef, pork or lamb in past 10 years	No	4	79			0.245
	Yes	166	1641	1.91	0.72–5.01	
How beef was eaten	Not eaten	9	131			0.346
	Rare	14	96	2.12	0.88–5.1	
	Medium	36	332	1.58	0.74–3.36	
	Well done	111	1163	1.39	0.69–2.8	
How pork was eaten	Not eaten	12	172			0.043
	Rare	2	3	9.56	1.46–62.76	
	Medium	6	25	3.44	1.19–9.98	
	Well done	150	1522	1.41	0.77–2.59	
How lamb was eaten	Not eaten	22	233			0.624
	Rare	1	6	1.77	0.20–15.33	
	Medium	18	134	1.42	0.74–2.75	
	Well done	129	1349	1.01	0.63–1.63	

RR, Relative risk; CI, confidence interval.

Table 2. Single variable analysis of preference for fresh and frozen food purchase in pregnant women asked to describe their shopping habits prior to their pregnancy

Food item	Toxoplasma seropositive	Toxoplasma seronegative	RR	95% CI	P value
Fresh beef mince	120/167	1132/1700	1.25	0.91–1.73	0.2
Frozen beef mince	60/167	595/1700	1.04	0.77–1.40	0.88
Fresh beef steak	124/160	1135/1631	1.46	1.02–2.08	0.05
Frozen beef steak	24/160	172/1631	1.44	0.96–2.16	0.11
Frozen beef roast	46/154	418/1638	1.22	0.88–1.69	0.28
Fresh beef roast	117/154	1230/1638	1.04	0.73–1.49	0.89
Fresh beefburger	70/158	569/1611	1.41	1.04–1.90	0.03
Frozen beefburger	73/158	908/1611	0.69	0.51–0.93	0.02
Fresh beef sausage	77/158	713/1630	1.20	0.89–1.62	0.26
Frozen beef sausage	67/158	742/1630	0.89	0.66–1.20	0.50
Fresh beef tongue	9/155	38/1638	2.29	1.25–4.20	0.02
Frozen beef tongue	2/155	7/1638	2.59	0.76–8.88	0.18
Fresh beef stew	94/159	850/1625	1.29	0.95–1.74	0.12
Frozen beef stew	67/159	759/1625	0.84	0.63–1.14	0.31
Fresh pork mince	30/163	264/1650	1.17	0.80–1.70	0.49
Frozen pork mince	13/163	152/1650	0.87	0.50–1.49	0.70
Fresh pork roast	108/159	1084/1608	1.02	0.74–1.40	0.97
Frozen pork roast	38/159	336/1608	1.17	0.83–1.65	0.43
Fresh pork chops	107/163	1028/1627	1.10	0.81–1.50	0.59
Frozen pork chops	42/163	386/1627	1.10	0.79–1.54	0.63
Fresh pork sausage	105/160	889/1613	1.50	1.09–2.04	0.01
Frozen pork sausage	79/160	800/1613	0.99	0.74–1.33	0.98
Fresh bacon	138/159	1368/1608	1.14	0.73–1.77	0.64
Frozen bacon	24/159	230/1608	1.06	0.70–1.60	0.88
Fresh pork stew	47/156	449/1593	1.09	0.79–1.51	0.67
Frozen pork stew	29/156	352/1593	0.82	0.56–1.21	0.36
Fresh lamb mince	40/162	340/1624	1.21	0.86–1.70	0.31
Frozen lamb mince	24/162	241/1624	1.00	0.66–1.51	0.92
Fresh lamb roast	100/161	967/1624	1.10	0.81–1.50	0.58
Frozen lamb roast	40/161	371/1624	1.11	0.79–1.55	0.63
Fresh lamb chops	90/163	746/1630	1.41	1.05–1.89	0.03
Frozen lamb chops	37/163	335/1630	1.12	0.79–1.59	0.59
Fresh turkey	115/137	1079/1343	1.25	0.81–1.94	0.37
Frozen turkey	55/137	608/1343	0.83	0.60–1.14	0.29
Fresh chicken	147/165	214/1623	1.22	0.76–1.95	0.48
Frozen chicken	80/165	720/1623	1.16	0.87–1.55	0.35

RR, Relative risk; CI, confidence interval.

eating various food items (categorized as never, once every few months, once a month, and once a week). How often beef tongue was eaten, how often lamb chops were eaten, how often beefburger was eaten and how often cured pork was eaten were found to be risk factors with *P* values <0.2 (results not shown). An initial multivariable logistic regression showed that location of childhood home and description of childhood home were significantly associated with toxoplasmosis. Three individuals whose ages were calculated as 100 years due to data entry error were assigned a missing age value. The number of observations used in the analysis increased from 1252

to 1705 in the final model (out of a possible 1897). The results of the final step can be seen in Table 3 and show that age, those feeding a dog raw meat, location of childhood home and description of childhood home were all significantly related to toxoplasmosis.

None of the two-way interactions were found to be significant.

#### Population-attributable fraction

Population-attributable fractions were calculated for significant risk factors. The highest positive values were associated with age >35 years (15.4%),

Table 3. Final multivariable logistic regression model analysing risk factors for toxoplasmosis in pregnant women in Kent (UK)

Parameter	OR	95% CI	P value
Constant	0.04	0.02–0.11	
Age (years)			
≤20	1.00		
20–24	1.65	0.61–4.49	
25–29	1.29	0.49–3.44	
30–34	1.30	0.49–3.43	0.004
35–39	3.00	1.10–7.91	
≥40	3.27	1.00–10.6	
Fed dog pieces of raw meat			
No	1.00		
Yes	1.78	1.14–2.75	0.014
Childhood home			
In the UK ≤50 miles away	1.00		
In UK >50 miles away	0.89	0.58–1.37	
In Europe, except UK	4.99	2.66–9.34	<0.001
Outside Europe	1.22	0.55–2.68	
In city or town with own garden	1.00		
In town, no garden	0.76	0.28–2.07	
In village	1.06	0.70–1.59	<0.001
In open countryside	2.94	1.77–4.89	
Attached to farm	4.04	2.06–7.93	

OR, Odds ratio; CI, confidence interval.

childhood home in Europe, excluding the United Kingdom (8.9%), a childhood home in the countryside (12%) or on a farm (7.1%) and feeding a dog raw meat (7.7%). However, the confidence limits for all of these exposure risks were wide.

#### Age and seroprevalence

Seropositivity increased with age from 5.6% in the 15–19 years age group to 16.7% in the 40–44 years age group (Table 4). The association of seropositivity with age >35 years was significant after multivariable analysis.

#### Cat and dog contact in childhood

Having a dog or cat in the home as a child was not a significant risk factor after multivariable logistic regression; however, the seroprevalence of toxoplasmosis was higher in childhood country/farm dwellers who lived with cats (39/148, 26.5%) than those with no cat (5/56, 8.9%). In contrast, those living with (35/158, 22%) and without (9/46, 19%)

Table 4. *Toxoplasma* seroprevalence by age in pregnant women in Kent (UK) October 1999 to November 2001\*

Age group (years)	Numbers	Seroprevalence (%)	95% CI
15–19	10/180	5.6	2.7–10.0
20–24	24/362	6.7	4.3–9.8
25–29	46/571	8.1	6.0–10.7
30–34	54/532	10.2	7.7–13.1
35–39	32/220	14.6	10.2–20.0
40–44	6/36	16.7	6.4–32.8

CI, Confidence interval.

\* Five women with ages outside the range 15–44 years are excluded from this table.

a dog during childhood, had similar rates of toxoplasmosis.

#### Gardening activity

No association was found between toxoplasmosis and type of garden (Table 1). A non-significant association [relative risk (RR) 1.26, 95% confidence interval (CI) 0.82–1.94] was found with toxoplasmosis and smoking while gardening. A  $\chi^2$  trend analysis showed no association between toxoplasmosis and either frequency of gardening activity or use of gloves while gardening. However, the prevalence of toxoplasmosis was lowest in the group who 'always used gloves' to handle soil (6/166, 3.6%).

#### Handling raw meat

No association was found between toxoplasmosis and handling raw meat in the kitchen. However, a non-significant association was found between smoking in the kitchen while handling raw meat and seropositivity (RR 1.85, 95% CI 0.82–4.16) (Table 1). Only 25 cases admitted to this risk factor. Indirect evidence implicating raw meat was provided by the significant association (RR 1.84, 95% CI 1.30–2.61) found between feeding a dog raw meat and toxoplasma seropositivity (Table 1). This association remained significant after multivariable logistic regression (Table 3). A similar question applied to feeding cats showed no association.

#### Consumption of food

Single variable analysis of cooking preferences for meats consumed showed only weak associations with

eating undercooked or medium-cooked pork that were not significant after logistic regression (Table 1).

Frequency of consumption of various foods was analysed using a  $\chi^2$  trend analysis. Associations between seroprevalence of toxoplasmosis and consumption of beefburger ( $P=0.018$ ), beef tongue ( $P=0.092$ ), lamb chops ( $P=0.085$ ) and cured pork products ( $P<0.001$ ) were found but were not significant after multivariable logistic regression.

### Preference for purchase of fresh or frozen food

Preference for purchasing fresh or frozen foods was analysed for 18 meat-containing products (Table 2). For 15 out of 18 products a preference for frozen purchase had a lower relative risk for toxoplasma seropositivity than the corresponding fresh product. The three products for which frozen purchase had a higher relative risk were represented by relatively low numbers (beef tongue, pork roast and lamb roast). In the single variable analysis, purchase of fresh beef mince, fresh beef steak, fresh beefburger, fresh beef tongue, fresh pork sausage and fresh lamb chops were associated with toxoplasma seroprevalence ( $P<0.20$ ) but were not found to be significant after multivariable logistic regression. No attempt was made to determine whether there was a statistically significant difference in risk associated with purchase of 'fresh' vs. 'frozen' produce because a valid method of comparison could not be identified.

## DISCUSSION

In this cross-sectional survey of pregnant women, we found that feeding a dog raw meat, living on a farm or in the countryside as a child, living in Europe excluding the United Kingdom as a child, and those aged  $\geq 35$  years of age were all significantly related to seropositivity for toxoplasmosis. Subgroup analysis suggests that living with cats may contribute to the higher toxoplasma seroprevalence in women whose childhood home was in the countryside or on a farm.

The overall prevalence of toxoplasma antibodies found (9.1%) by this study confirms previous reports of a decrease in UK seroprevalence since the 1960s [7, 13, 14] and is similar to that found in recent studies from the United Kingdom [9] and Norway [15]. In contrast seroprevalence rates found in France [16], Italy [17] and Greece [18] have been  $>40\%$ .

The association between toxoplasmosis and having lived overseas may reflect residence in countries with a higher incidence of toxoplasmosis and is consistent with the observation in a Norwegian study of higher seroprevalence in women with foreign names [19] and the association with country of birth and ethnic group found in a study of pregnant women in London [8]. Previous studies have usually reported higher rural prevalence of toxoplasmosis [20–22] as found by this study but higher urban rates have also been reported and may represent rates in more cosmopolitan [8] or non-European populations [23, 24]. In this study where none of the population centres exceed 100 000, the rates may reflect true rural–urban differences rather than migrant population characteristics. We were unable to demonstrate a risk through handling raw meat in the kitchen but did find an association with feeding a dog raw meat. A previous study has found an association with dog contact [25] which the authors speculated may have been due to contamination of the dog's coat by oocysts from cat faeces. Our findings provide an additional explanation.

The absence of significant dietary risk factors is perhaps the most striking finding in our study and is in contrast to a recent multi-centre European case-control study which found that 30–60% of recent infections were associated with consumption of undercooked or cured meat products and approximately 6–17% with soil contact [26]. An independent study from Naples [17] also identified consumption of cured pork and raw meat products as the most significant risk factors for recent toxoplasmosis in a pregnant population. We believe that the differences between our results and these recent European reports are explained by the contrasting study designs combined with the lower food-related incidence of toxoplasmosis in the United Kingdom. In contrast to the European multi-centre study [26] which looked only at newly acquired toxoplasma infection as evidenced by the presence of IgM-class antibody we have studied all women who were seropositive regardless of the timing of infection. This has enabled identification of risk factors operating during childhood at the expense of providing less information about risk factors associated with recent infection. The Naples study was closer in design to our study in that questionnaires investigating exposure risk were completed by all participants, however, the range of questions was relatively restricted and may not have permitted the detection of childhood influences on seropositivity.

Our study suggests that in a low-prevalence country such as the United Kingdom, childhood environmental exposure is a major determinant of seropositivity in early adult life. Contamination of that childhood environment through ownership of cats provides a plausible explanation for some of the association found with country and farm home location. However, our questionnaire did not examine all of the possible mechanisms of soil and environmental exposure and was not able to determine whether the direct soil contact (contaminated by cat oocysts) or indirect contact through consumption of soil-contaminated fruit or vegetables as described by Kapperud [19] provides an explanation for the association. It is also possible that recall problems and our failure to ask specific questions about childhood diet, have biased our analysis towards incriminating environmental rather than dietary factors in childhood acquisition of toxoplasmosis.

While no statistically significant food associations were found, the single variable analyses provided information on trends which are consistent with recent European studies implicating undercooked meat and cured pork products [17, 19, 26]. Larger studies may be required to identify the lower UK food-related incidence of toxoplasmosis. The non-significant associations with cured pork products and beef tongue were particularly striking despite the relatively low consumption rates in our population. These findings highlight the potential for food-related toxoplasmosis in pregnancy to dramatically increase, if dietary habits change in response to increased travel and exposure to more exotic foods while overseas.

Our study has also attempted to explore the hypothesis mentioned by others [27, 28], that the increased use of frozen produce has contributed to the decline of toxoplasmosis. We have observed a generally lower risk of toxoplasmosis associated with purchase of frozen as opposed to fresh produce but have not been able to show that this difference is statistically significant. It is possible that confounding variables explain the non-significant associations found.

#### **Implications for future studies**

The knowledge gained from this and other recent European studies illustrates that epidemiological studies carried out in antenatal populations can elucidate sources of toxoplasma infection provided that appropriate data is collected. More detailed analysis of food sources in the United Kingdom will require

much larger studies to compensate for the low incidence of primary infection. It will be important for any such studies to analyse all known risk factors for toxoplasmosis including poor kitchen hygiene and consumption of contaminated fruit and vegetables which were omitted from this study.

#### **Implications for antenatal care**

The value of prenatal screening for toxoplasma infection remains controversial and has recently been the subject of a detailed review [29]. The high cost and limited benefit of introducing routine antenatal screening in a low-prevalence country such as the United Kingdom had been identified as early as 1984 [30] and led to a decision to base control of toxoplasma in pregnancy on health education rather than screening. This study provides no evidence to suggest that the overall seroprevalence of toxoplasmosis in pregnancy is increasing in the United Kingdom. Consequently current advice to pregnant women on the avoidance of contact with soil contaminated by cat faeces, consumption of raw or undercooked meats and unwashed fruit and vegetables remains appropriate. However, the low but increasing age-related seroprevalence in women of childbearing age, found in both this study and a recent study from Yorkshire [9] demonstrates the need to have systems in place which can detect any increase in toxoplasmosis that might result from changes in the national diet or childbearing age. This could be achieved through targeted screening of antenatal blood samples or other banks of stored serum samples. Linkage of such studies to dietary surveys in pregnant women may be more cost-effective than independent research.

#### **CONCLUSION**

Risk factors for toxoplasmosis present in childhood contributed significantly to the seroprevalence found in this UK antenatal population. Location of the childhood home in Europe excluding the United Kingdom or in a countryside/farming setting and feeding a dog raw meat were significantly associated with toxoplasma seropositivity. Evidence was also found that having a cat/kitten in the home may account for some of the higher prevalence associated with the childhood home. Large multi-centre studies will be needed to recruit sufficient cases of primary infection to identify dietary sources in the United Kingdom. Most pregnant women in the United Kingdom are

susceptible to toxoplasmosis and, therefore, continuing education of women about dietary and environmental sources of infection remains essential.

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