Sources of toxoplasma infection in pregnant women: European multicentre case-control study

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Abstract

Objective To determine the odds ratio and population attributable fraction associated with food and environmental risk factors for acute toxoplasmosis in pregnancy.
Design Case-control study.
Setting Six large European cities.
Participants Pregnant women with acute infection (cases) detected by seroconversion or positive for anti-*Toxoplasma gondii* IgM were compared with pregnant women seronegative for toxoplasma (controls).
Main outcome measures Odds ratios for acute infection adjusted for confounding variables; the population attributable fraction for risk factors.
Results Risk factors most strongly predictive of acute infection in pregnant women were eating undercooked lamb, beef, or game, contact with soil, and travel outside Europe and the United States and Canada. Contact with cats was not a risk factor. Between 30% and 63% of infections in different centres were attributed to consumption of undercooked or cured meat products and 6% to 17% to soil contact.
Conclusions Inadequately cooked or cured meat is the main risk factor for infection with toxoplasma in all centres. Preventive strategies should aim to reduce prevalence of infection in meat, improve labelling of meat according to farming and processing methods, and improve the quality and consistency of health information given to pregnant women.

Introduction

In Europe, congenital toxoplasmosis affects between 1 and 10 in 10 000 newborn babies,[1] of whom 1% to 2% develop learning difficulties or die and 4% to 27% develop retinochoroidal lesions leading to permanent unilateral impairment of vision.[2–6] Effective prevention of congenital toxoplasmosis depends on avoidance of infection during pregnancy. Infection is acquired by ingestion of viable tissue cysts in meat or oocysts excreted by cats that contaminate the environment.[7] Uncertainty about how most women acquire infection results in advice to avoid numerous risk factors, making compliance difficult.[8 9] Development of more focused strategies requires up to date and regionally relevant information on the principal sources of infection during pregnancy.

The prevalence of previous toxoplasma infection in pregnant women ranges from 10% in the United Kingdom[10] and Norway[11] to around 55% in France[12] and Greece[13]; in many countries it has declined sharply over the past three decades.[14–16] Regional variation has been attributed to climate,[11] cultural differences in the amount and type of raw meat consumed,[7 17] and the increased consumption of meat from animals farmed indoors and frozen meat.[7 18] The decline in prevalence of infection, however, does not necessarily reflect a fall in the incidence of toxoplasmosis acquired during pregnancy. Instead, the decline in prevalence in pregnant women probably reflects a decline in incidence during childhood.[15] More women are susceptible to infection now, and the frequency of exposure to risk factors for infection may have increased. Recent changes include a shift from consumption of beef to pork and poultry and increased consumption of organic meat and "value added" products such as ready meals and burgers.[19] These trends may have increased exposure to *Toxoplasma gondii* as pork and lamb carry a higher risk of infection than beef or poultry. Animals that are reared outdoors may be at greater risk of environmental exposure than animals reared indoors.

The most appropriate measure for ranking the principal risk factors for infection is the population attributable fraction, which indicates the proportionate reduction in infection that would be achieved if pregnant women were entirely unexposed to a factor compared with their current pattern of exposure.[20] Population attributable fractions for food and environmental exposures may vary between regions and have previously been reported in two small studies, which are not directly comparable.[21 22]

We compared the proportion of incident cases that could be attributed to identified risk factors in six European centres. We used a standardised case-control study design involving comparison of exposures in acutely infected and susceptible pregnant women.

Methods

The centres involved in the study—Naples, Lausanne, Copenhagen, Oslo, Brussels, and Milan—operate screening for toxoplasmosis. In five centres, women were prospectively identified by prenatal screening. In Copenhagen, women were identified postnatally by testing neonatal Guthrie card blood spots for toxoplasma specific IgG and investigation of stored prenatal serum samples.[6] Cases were pregnant women diagnosed with acute toxoplasma infection between January 1994 and June 1995, on the basis of seroconversion (change from a negative to positive result for antibodies specific for toxoplasma) or detection of IgG and IgM specific for toxoplasma (with the immunoglobulin-M immunosorbent agglutination assay (ISAGA)[23] or immunofluorescent antibody test IFAT[24]) and rising IgG titre, low avidity of specific IgG, or presence of IgA antibodies. The controls were the next four women negative for IgG, who were tested with the same screening test in the same laboratory, after the date of the positive test result that identified

the case. Women who were referred from outside the population offered routine screening were excluded.

Data were collected by interview soon after diagnosis of infection by using a standard questionnaire translated into the local language. Interviews were conducted postnatally in Copenhagen and in other centres if delays occurred. All questions related to exposure before the test date when the woman was categorised as case or control. Interviews were by telephone except in Lausanne, where cases and some controls were interviewed in the clinic. All women and their interviewers were aware of the woman's infection status.

Information collected at interview
Women were first asked how they could avoid toxoplasma infection to assess their knowledge about sources of infection. The first three answers were recorded. They were then asked about age, parity, educational level, foreign travel, high risk occupations, environmental exposures, contact with cats, diet, and consumption of untreated water or provision of piped water. Consumption of "raw or undercooked meat," "cooked meat," "raw sausage," "locally produced dry cured meat" and "salami," and "tasting raw meat while cooking" was coded on four levels: not in the past four months; less than weekly; weekly; and daily. The same categories were used for "cleaning up cat faeces" and "working in the field or garden with your hands in the soil."

Analysis
We used a multiplicative, unconditional logistic regression model, allowing for centre, maternal age, and interval between diagnosis and interview to examine the risk associated with each exposure. Models that conditioned on centre or case-control set gave virtually identical results.

Secondly, we included variables with a P value of ≤ 0.25 in a multivariate, multiplicative, logistic regression model to allow for potential confounding (see table 2). As public health recommendations would be for avoidance rather than reducing exposure during pregnancy, binary variables were grouped as any exposure in the past four months (categories 2-4) compared with none (category 1).

Thirdly, we estimated the population attributable fraction according to the method described by Coughlin et al.[25] Variables with a P value of ≤ 0.25 were included in an additive logistic regression model in which risks associated with different exposures combine in an additive manner. This is biologically more plausible than a multiplicative model and has the advantage that the population attributable fraction for all exposures combined is equal to the sum of the population attributable fractions for individual exposures.

Finally, we calculated the effect of knowledge of risk factors mentioned by women at the start of interview on avoidance of exposure, adjusted for maternal age, centre, and interval between diagnosis and interview.

Results

A total of 252 infected women (cases) and 858 control women were enrolled; 150 eligible control women did not complete an interview because of contact failure, inability to speak the local language, or refusal to par-

ticipate. The numbers of participants (cases, controls) were 99 and 341 in Naples; 37 and 147 in Lausanne; 41 and 118 in Copenhagen; 34 and 108 in Oslo; 22 and 88 in Brussels; and 18 and 57 in Milan. As the odds ratios were similar for infected women defined by seroconversion or IgM positivity, these groups were combined for further analyses.

Control women were 0.8 years older than infected women (mean ages 28.6 and 27.8 years, respectively; P = 0.02). The interval between the test that defined control status or diagnosis of infection and interview was similar in control women (median (interquartile range) 52 (17-115) days) and infected women (46 (11-113) days; P = 0.33).

Multiplicative models
Table 1 shows the odds ratio for each exposure adjusted for centre, maternal age, and interval between diagnosis of infection and interview. No significant associations were detected between infection and presence of cats (whether adult or kittens), the diet and hunting habits of the cats, or cleaning a cat's litter tray. Soil contact was associated with a twofold increased risk of infection, as was working with animals (on farms, in an abattoir, or with meat as a butcher or cook) and travel outside Europe or the United States and Canada. Infection was also associated with drinking untreated water or having no piped water but not with living on a farm.

The risk of toxoplasma infection was increased in women who reported tasting meat while preparing meals or eating raw or undercooked beef, lamb, or "other" meats, predominantly game (table 2), but not pork. There was a significant trend (P < 0.01) in the risk of infection with the frequency of consumption of undercooked lamb or beef, salami, dried cured pork, or raw sausage. Consumption of unpasteurised milk or milk products was also associated with infection.

After we allowed for the confounding effects of all exposures with a P value of ≤ 0.25, consumption of raw or undercooked beef, lamb, or "other" meat, tasting raw meat while cooking, working with animals, contact with soil, and travel outside Europe or the United States and Canada were significantly associated with toxoplasma infection (table 2). There was no evidence that these risks varied between centres or regional groupings of Scandinavia, central Europe (Brussels and Lausanne), and Italy (all tests for interaction P > 0.3).

Population attributable fraction
Between 30% and 63% of infections in the different centres could be attributed to meat consumption, although the type of meat differed (table 3). Eating lamb and "other meat" was more important in northern and central European centres than in Italy. The proportion of infections attributed to eating salami was 10% to 14% in Milan, Naples, and Brussels and 3-5% elsewhere.

In Italy, 6-7% of infections were attributed to soil contact compared with 16-17% elsewhere. None to 9% of the infections were attributed to travel outside Europe or the United States and Canada. In Lausanne, 14% of infections were attributed to consumption of unpasteurised milk or milk products, whereas elsewhere the population attributable fraction was $\leq 5\%$. In all centres a large proportion of

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Table 1 Risk factors for *Toxoplasma gondii* infection adjusted for age, location, and period between diagnosis of infection and interview in 252 infected women and 852 control women*

Exposure	Cases		Controls		Odds ratio (95% CI)	P value
	Exposed	Not exposed	Exposed	Not exposed		
Cat						
Kitten in home	14	233	28	793	1.4 (0.7 to 2.8)	0.31
Adult cat in home	36	210	110	703	1.0 (0.6 to 1.5)	0.89
Cat and kitten in home	8	238	14	807	1.7 (0.7 to 4.1)	0.28
Any cat in home	42	206	124	697	1.0 (0.7 to 1.5)	0.99
Cleaning litter tray	26	214	65	748	1.3 (0.8 to 2.1)	0.37
Cat that hunts	23	230	47	752	1.6 (0.9 to 2.7)	0.12
Cat fed raw meat	14	225	43	763	1.0 (0.5 to 1.9)	0.97
Cat fed tinned food	37	208	113	705	1.0 (0.6 to 1.5)	0.81
Meat						
Cooked meat <1/week†	27	7	79	18	0.8 (0.3 to 2.1)	0.59
Cooked meat ≥1/week†	212		725		0.8 (0.3 to 1.9)	0.54
Raw sausage <1/week†	22	210	60	745	1.2 (0.7 to 2.0)	0.61
Raw sausage ≥1/week†	9		8		3.2 (1.2 to 9.0)	0.02‡
Dry to cured meat <1/week†	64	136	202	518	1.2 (0.8 to 1.7)	0.36
Dry to cured meat ≥1/week†	42		90		1.8 (1.2 to 2.8)	0.01‡
Salami <1/week†	76	47	297	187	1.0 (0.7 to 1.6)	0.89
Salami ≥1/week†	125		335		1.6 (1.1 to 2.4)	0.02‡
Raw/undercooked beef	103	140	181	636	2.4 (1.6 to 3.4)	<0.001§
Raw/undercooked lamb	19	220	17	795	3.2 (1.5 to 6.9)	0.002§
Raw/undercooked pork	21	219	33	779	1.6 (0.8 to 3.0)	0.18
Other raw/undercooked meat	15	225	11	801	3.9 (1.6 to 9.5)	0.003
Frozen meat	207	39	715	103	0.8 (0.5 to 1.2)	0.22
Taste meat cooking <1/week†	52	182	88	717	2.3 (1.5 to 3.4)	<0.001
Taste meat cooking ≥1/week†	14		13		4.7 (2.1 to 10.9)	<0.001§
Other "food related" exposures						
Unpasteurised milk	36	210	75	746	1.8 (1.1 to 2.9)	0.01
Untreated water	53	194	123	697	1.7 (1.1 to 2.6)	0.02
Use of microwave cooker	41	206	101	718	1.3 (0.8 to 2.2)	0.24
Other "lifestyle" exposures						
Contact with soil	109	139	267	553	1.9 (1.4 to 2.8)	<0.001‡
Working with animals	30	218	54	767	1.9 (1.1 to 3.0)	0.01§
Travel outside Europe/US or Canada	29	220	49	759	2.2 (1.3 to 3.6)	0.002
Living on farm	23	229	47	811	1.6 (0.9 to 2.7)	0.11

*Numbers do not always add up to total as some women did not answer all questions.
†Compared with no exposure in past four months.
χ^2 test for trend across all five levels of exposure (never, exposed but not in past four months, monthly, weekly, or daily): ‡P<0.01; §P<0.001.

infection (14% to 49%) remained unexplained by the variables included in the model.

Table 2 Multivariate analysis of risk factors for *Toxoplasma gondii* infection adjusted for age, location, period between diagnosis of infection and interview, and all other exposures shown

Exposure	Odds ratio (95% CI)	P value
Cat that hunts	1.26 (0.7 to 2.4)	0.47
Eat raw sausage	0.91 (0.5 to 1.6)	0.76
Eat dry to cured meat	0.82 (0.7 to 1.4)	0.99
Eat salami*	1.31 (0.9 to 2.0)	0.22
Eat frozen meat	0.77 (0.5 to 1.2)	0.29
Raw/undercooked beef*	1.73 (1.1 to 7.2)	0.01
Raw/undercooked lamb*	3.13 (1.4 to 7.2)	0.007
Raw/undercooked pork*	1.40 (0.7 to 2.8)	0.34
Other meat*†	4.12 (1.6 to 10.9)	0.004
Taste meat cooking*	1.52 (1.0 to 2.4)	0.07
Unpasteurised milk*	1.47 (0.9 to 2.5)	0.16
Untreated water	1.21 (0.7 to 2.0)	0.46
Use of microwave cooker	1.30 (0.8 to 2.3)	0.35
Contact with soil*	1.81 (1.2 to 2.7)	0.005
Working with animals*	1.50 (0.8 to 2.7)	0.19
Travel outside Europe/US or Canada*	2.33 (1.3 to 4.1)	0.003
Living on farm	1.15 (0.6 to 2.2)	0.66

*Included in additive relative risk model used to estimate population attributable fraction.
†Other meat includes venison, horse, rabbit, whale, and game birds.

Knowledge of risk factors

Susceptible control women listed contact with cats, eating raw meat, and eating raw or unwashed fruit or vegetables (table 4) as the main factors that could cause infection with *T gondii*. Few women mentioned contact with soil.

Infected women and control women showed similar associations between factors mentioned and reported exposure to raw meat, cats, or soil so the odds ratios were estimated from the combined data. Women who mentioned raw or undercooked meat as a risk factor were less likely to report eating or tasting raw or undercooked meat (adjusted odds ratio 0.6; 95% confidence interval 0.4 to 0.9). Those who mentioned cats were more likely to have a cat in the home (adjusted odds ratio for cats 2.4; 1.5 to 3.8), whereas those who mentioned soil were not significantly more likely to be exposed to soil (1.7; 0.9 to 3.2).

Discussion

Risk factors that most strongly predicted acute infection in pregnant women were eating raw or undercooked lamb, beef, or "other" meat, contact with soil and travel outside Europe or the United States and Canada. Weaker associations, not significant at the 5%

Table 3 Population attributable fraction for toxoplasma infection by exposure in pregnant women in six European centres. Figures are numbers (percentage) of infected women (cases)

Centre (No of cases)	Salami	Beef	Lamb	Other meat	Tastes meat	Milk	Soil	Works with animals	Travel	Unknown
Naples (99)	14 (14)	14 (14)	1 (1)	1 (1)	6 (6)	4 (4)	6 (6)	2 (2)	6 (6)	46 (46)
Milan (18)	2 (11)	2 (11)	0	1 (5)	1 (3)	1 (1)	1 (7)	2 (13)	0	9 (49)
Copenhagen (41)	2 (4)	11 (27)	3 (8)	1 (2)	4 (9)	2 (5}	7 (17)	3 (7)	1 (3)	7 (18)
Oslo (34)	1 (3)	6 (19)	7 (21)	5 (16)	1 (3)	0	6 (17)	1 (2)	2 (5)	5 (14)
Brussels (22)	2 (10)	1 (6)	2 (10)	2 (7)	1 (3)	1 (3)	4 (16)	1 (4)	2 (9)	7 (32)
Lausanne (37)	2 (5)	3 (8)	4 (10)	5 (13)	0	5 (14)	6 (16)	1 (3)	3 (9)	8 (22)

level, were observed for tasting raw meat during preparation of meals, eating salami, drinking unpasteurised milk, and working with animals. Contact with cats, kittens, cats' faeces, or cats who hunt for food was not a risk factor for infection.

The association between eating raw or undercooked meat and acute toxoplasma infection has been a consistent finding in previous studies. The types of meat, however, have varied. In a Norwegian study, undercooked lamb and pork but not beef were identified as risk factors,[22] whereas in northern France beef or lamb but not pork were risk factors.[26] Consumption of cured pork products was investigated in two studies, and both found a strong association with infection.[21 26]

Evidence from studies that used bioassays suggests that lamb, goat, pork, and game are more commonly infected than beef and that chicken rarely contains viable cysts.[27–30] The risk of infected meat also depends on the age of the animal, the proportion of time the animal spent indoors,[27] farm hygiene,[27 31] and the specific tissues used: non-skeletal muscle (heart, diaphragm, and tongue) has a higher density of cysts than skeletal muscle.[32 33] Findings from biological studies can help to explain our results. Firstly, most pork is produced from pigs reared indoors and some would have been frozen. As freezing kills cysts the risk associated with eating raw or undercooked pork would be attenuated. Secondly, the pig meat used in salami is more likely to be infected because it includes non-skeletal muscle and may be derived from older animals farmed outdoors. The first report of the isolation of viable *T gondii* from one of 67 samples of ready-to-eat cured meat in the United Kingdom[34] showed that curing methods may not kill all tissue cysts[35] or may not be stringently applied.

Thirdly, although *T gondii* is rarely isolated from beef,[32] the large amount consumed may explain the strong association with infection. Conversely, the association with tasting meat while cooking, which implies consumption of small quantities, may be due to tasting minced meat, which includes beef, lamb, or pork meat. The association between infection and unpasteurised milk or milk products was unexpected. *T gondii* tachyzoites have been isolated from goats' milk and cows' colostrum[28] but are destroyed within minutes by gastric juices.[7] The association might be due to oocyst contamination by dirty production techniques or to confounding by other lifestyle factors (for example, eating undercooked organically produced meat).

Contact with soil or vegetables or fruit contaminated with soil was identified as a risk factor for toxoplasma infection in pregnancy in two of three studies that adjusted for confounders.[21 22 26] Cats excrete oocysts (up to 10 million oocysts per day[7]) for

only two weeks of their life, when they first acquire infection. Oocysts become infective one to five days after excretion, are spread by surface water, and can survive for more than a year.[28] Thus contact with soil and water, rather than direct contact with cats, is a risk factor for infection. The lack of an association with cat contact was also reported by two previous studies.[22]

Contact with cats was often mentioned as a risk factor whereas soil contact was rarely mentioned. Although we cannot exclude the possibility of recall bias, the negative and positive associations with cat and soil contact suggest that recall bias did not have a major effect. In addition, poor recall of exposures or varying interpretation of our questions may have attenuated the observed associations.

Population attributable fraction

Estimation of the population attributable fraction assumes that exposures have a net causal effect.[20] We specified, a priori, a P value of ≤0.25 for inclusion of exposures "causally" associated with infection after adjustment for all relevant exposures. If the P value is set higher the population attributable fraction may be overexplained by irrelevant factors. If set too low some causal exposures may be excluded. Given our criteria, we failed to explain between 14% to 49% of infections in the different centres. The risk of infection after exposure to a risk factor did not vary significantly with centre, but the proportion of cases that could be attributed to each exposure did vary.

Public health action

The single most important health message for pregnant women in all centres in the study is to avoid eating any meat that has not been thoroughly cooked. The importance of other risk factors varied between centres. Consequently, advice to ensure that all fruit and vegetables are thoroughly washed and to avoid soil contact, working with animals, or drinking unpasteur-

Table 4 Factors mentioned by control women when asked how to avoid toxoplasmosis.* Figures are percentages of women

	Naples (n=341)	Milan (n=57)	Oslo (n=108)	Brussels (n=88)	Lausanne (n=147)	Total (n=741)
No factors mentioned	51	9	22	2	10	29
Cat contact	36	44	70	70	52	49
Raw meat	44	77	54	89	66	58
Soil contact	1	0	14	14	6	6
Raw/unwashed vegetables and fruit	43	51	5	31	49	38
Good personal/kitchen hygiene	1	5	9	39	1	7
Other	6	7	4	5	4	5
Raw meat, cat contact, and vegetables/fruit	29	25	3	20	32	24

*Questions not asked in Copenhagen.

> ### What is already known on this topic
>
> Eating undercooked meat or cured meat is a risk factor for toxoplasma infection
>
> Contact with cats is not a risk factor for infection as excretion of oocysts is limited to only a few weeks
>
> ### What this study adds
>
> In six European centres eating undercooked, raw, or cured meat contributed to between 30% and 63% of infections, with soil contact contributing to up to 17% of infections
>
> Action to reduce infection rates should include improved information about the risk associated with undercooked or cured meat, labelling of meat according to farming and processing methods, and measures to reduce infection in domestic animals

ised milk may be warranted but the advantages of comprehensive information must be balanced against the diminished emphasis on meat. Our results may not be generalisable to countries with different climates, farming, or culinary practices, particularly outside Europe, for which we recommend that local case-control studies are carried out to identify the main risk factors.

Although health information should be regarded as a right,[36] many obstetric units have no policy on advice or information about toxoplasmosis.[37-39] Where it is offered, information is often inconsistent,[9 37] and ignorance, even among women in centres operating prenatal screening, is widespread.[40] In a French study, only half of the susceptible women knew of more than one risk factor for toxoplasma infection, and 11% could not cite any.[41] In our study, the proportion of women who could not cite any risk factors ranged from 2% in Brussels to 51% in Naples. A further concern is that knowledge may not lead to avoidance of exposure. In our study, lower rates of exposure were observed among women who mentioned raw meat as a risk factor but not among those who mentioned soil. Health promotion strategies must be based on an understanding of factors affecting women's behaviour.[42] Information given by clinicians,[26 43] within groups,[44] or via the media[45] may be more effective than written material.

Avoidance of infected meat could reduce the risk of infection during pregnancy by between 30% and 63%. To achieve this, health protection should be considered together with health promotion. Potential strategies include improved labelling of the source of meat and the type of processing (for example, farmed indoors or frozen) and measures to reduce infection in domestic animals (for example, improved farm hygiene).[27]

Although our study focused on pregnant women, postnatally acquired toxoplasmosis is an important cause of eye disease[46] and can be fatal in immuno-compromised patients.[47] Health information and health protection strategies may be as relevant to the general public as they are to pregnant women.

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