

Risk Factors for *Toxoplasma gondii* Infection in the United States

Jeffrey L. Jones,¹ Valerie Dargelas,² Jacquelin Roberts,¹ Cindy Press,² Jack S. Remington,^{2,3} and Jose G. Montoya^{2,3}

¹Division of Parasitic Diseases, National Center for Zoonotic, Vectorborne, and Enteric Diseases, Coordinating Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; ²Palo Alto Medical Foundation, Toxoplasma Serology Laboratory, Palo Alto, and ³Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, California

Background. Toxoplasmosis can cause severe ocular and neurological disease. We sought to determine risk factors for *Toxoplasma gondii* infection in the United States.

Methods. We conducted a case-control study of adults recently infected with *T. gondii*. Case patients were selected from the Palo Alto Medical Foundation Toxoplasma Serology Laboratory from August 2002 through May 2007; control patients were randomly selected from among *T. gondii*-seronegative persons. Data were obtained from serological testing and patient questionnaires.

Results. We evaluated 148 case patients with recent *T. gondii* infection and 413 control patients. In multivariate analysis, an elevated risk of recent *T. gondii* infection was associated with the following factors: eating raw ground beef (adjusted odds ratio [aOR], 6.67; 95% confidence limits [CLs], 2.09, 21.24; attributable risk [AR], 7%); eating rare lamb (aOR, 8.39; 95% CLs, 3.68, 19.16; AR, 20%); eating locally produced cured, dried, or smoked meat (aOR, 1.97; 95% CLs, 1.18, 3.28; AR, 22%); working with meat (aOR, 3.15; 95% CLs, 1.09, 9.10; AR, 5%); drinking unpasteurized goat's milk (aOR, 5.09; 95% CLs, 1.45, 17.80; AR, 4%); and having 3 or more kittens (aOR, 27.89; 95% CLs, 5.72, 135.86; AR, 10%). Eating raw oysters, clams, or mussels (aOR, 2.22; 95% CLs, 1.07, 4.61; AR, 16%) was significant in a separate model among persons asked this question. Subgroup results are also provided for women and for pregnant women.

Conclusions. In the United States, exposure to certain raw or undercooked foods and exposure to kittens are risk factors for *T. gondii* infection. Knowledge of these risk factors will help to target prevention efforts.

In the United States, among the ~4.2 million live births per year, congenital *Toxoplasma gondii* infection occurs in ~500 to 5000 newborns [1–3]. In addition, in the United States ocular toxoplasmosis affects an estimated 1.26 million persons [4–6], 22.5% of the adolescent and adult population is infected with the organism [5], and ~89% of women of childbearing age are susceptible to acute infection with *T. gondii* [7] and are at risk of transmitting the parasite to their offspring if they acquire a primary infection during gestation [8]. Con-

genital toxoplasmosis caused ocular disease in 12%–30% of children prospectively followed up by serial ophthalmologic evaluations [9–12], with new ocular lesions occurring in up to 31% of referred children who were treated and followed up to a mean age of 10.8 years [13]. In addition, postnatal infection is now thought to cause more cases of ocular toxoplasmosis than congenital infection [4]. Congenital infection has also been shown to result in intracranial calcifications in 9.5% of infants identified by prenatal screening programs and in 21.7% of infants identified by postnatal programs [14]. Other manifestations of congenital disease include hydrocephaly, microcephaly, and psychomotor and mental retardation. In immunocompromised patients, including those with organ transplants, AIDS, and cancer, and in those taking immunosuppressive drugs, reactivated and untreated toxoplasmosis has a high mortality rate.

Prevention of *T. gondii* infection depends on avoidance of the organism in cat feces, soil, water, and food, including undercooked meat [15]. Using a standardized case-control design and laboratory testing techniques

Received 21 January 2009; accepted 28 April 2009; electronically published 10 August 2009.

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Department of Health and Human Services or the Centers for Disease Control and Prevention.

Reprints or correspondence: Dr Jeffrey L. Jones, Mailstop F-22, Div of Parasitic Diseases, National Center for Zoonotic, Vectorborne, and Enteric Diseases, Coordinating Center for Infectious Diseases, Centers for Disease Control and Prevention, 4770 Buford Hwy, Atlanta, GA 30341 (jjj1@cdc.gov).

Clinical Infectious Diseases 2009;49:878–84

© 2009 by the Infectious Diseases Society of America. All rights reserved.
1058-4838/2009/4906-0008\$15.00
DOI: 10.1086/605433

Table 1. Demographic Characteristics of Case and Control Patients—*Toxoplasma gondii* Case-Control Study, United States, August 2002 through May 2007

Characteristic	Proportion (%)		<i>P</i> ^b
	Case patients ^a	Control patients	
Age			<.01
18–29 years	49/148 (33.1)	140/413 (33.9)	
30–49 years	81/148 (54.7)	259/413 (62.7)	
≥50 years	18/148 (12.2)	14/413 (3.4)	
Sex			<.01
Male	22/148 (14.9)	18/412 (4.4)	
Female	126/148 (85.1)	394/412 (95.6)	
Race			.58
White	125/148 (84.5)	328/408 (80.4)	
Black	5/148 (3.4)	18/408 (4.4)	
Asian/Pacific Islander	7/148 (4.7)	32/408 (7.8)	
Other	11/148 (7.4)	30/408 (7.4)	
Ethnicity			.47
Hispanic	8/146 (5.5)	29/400 (7.2)	
Non-Hispanic	138/146 (94.5)	371/400 (92.8)	
Education			.09
High school or less	25/148 (16.9)	43/406 (10.6)	
Some college	30/148 (20.3)	74/406 (18.2)	
College graduate	93/148 (62.8)	289/406 (71.2)	
Region of United States ^c			.14
Northeast	66/148 (44.6)	142/413 (34.4)	
South	28/148 (18.9)	102/413 (24.7)	
Midwest	23/148 (15.5)	64/413 (15.5)	
West	31/148 (21.0)	105/413 (25.4)	

^a Among case patients, 2 did not indicate ethnicity; among control patients, 1 did not indicate sex, 5 did not indicate race, 13 did not indicate ethnicity, and 7 did not indicate education.

^b Any differences between case and control patients.

^c Regions were defined as follows: the Northeast included Connecticut, Maine, Massachusetts, New Hampshire, Rhode Island, Vermont, New Jersey, Pennsylvania, and New York; the Midwest included Indiana, Illinois, Michigan, Ohio, Wisconsin, Iowa, Kansas, Minnesota, Missouri, Nebraska, North Dakota, and South Dakota; the South included Delaware, Florida, Georgia, Maryland, North Carolina, South Carolina, Virginia, West Virginia, Alabama, Kentucky, Mississippi, Tennessee, Arkansas, Louisiana, Oklahoma, Texas, and Washington, DC; and the West included Arizona, Colorado, Idaho, New Mexico, Montana, Utah, Nevada, Wyoming, Alaska, California, Hawaii, Oregon, and Washington.

available at the Palo Alto Medical Foundation *Toxoplasma* Serology Laboratory (PAMF-TSL) (<http://www.pamf.org/serology/>), we conducted a study to determine the risk factors for recent *T. gondii* infection and the proportion of recent *T. gondii* infections that could be attributable to each of the factors identified.

METHODS

The *Toxoplasma* case-control study was conducted by the PAMF-TSL in collaboration with the Centers for Disease Control and Prevention (CDC). Case patients were defined as persons ≥18 years of age from the United States who were referred for toxoplasmosis evaluation to the PAMF-TSL and were found to have been infected with *T. gondii* within 6 months before

the date the serum sample was obtained [16]. The patients' serum samples were sent by their primary physicians (from throughout the United States) to the PAMF-TSL for reference laboratory testing from August 2002 through May 2007 because of positive *Toxoplasma* immunoglobulin G (IgG) and immunoglobulin M (IgM) test results obtained at a clinical (non-reference) laboratory and/or clinical symptoms suggestive of toxoplasmosis. For each of the case patients, confirmatory testing performed at the PAMF-TSL revealed that the patient had acquired their primary infection within 6 months of serum sampling (see the section on serological tests below for a description of confirmatory testing) [16, 17]. Control patients were randomly selected from among *T. gondii*-seronegative persons tested at the PAMF-TSL within the year before each identified case patient. An attempt was made to identify 2 or 3 persons as control patients from the same state as each case patient. If control patients were not available from the same state, selection of control patients in the same geographical region of the case patient was attempted (for definitions of geographical regions, see the footnotes of table 1).

Questionnaire. The survey instrument was developed from a standardized questionnaire [18] with input from physicians, epidemiologists, veterinarians, health educators, and laboratorians at the CDC and the PAMF-TSL. The questionnaire was pilot tested by 4 case patients and 4 control patients identified through the PAMF-TSL. Questionnaires were mailed to the case patients' or control patients' physicians requesting their (and their patients') participation. After informed consent was obtained, participants were asked to complete the self-administered questionnaire. The exposure variables evaluated a comprehensive set of factors. The following is a partial list: educational level (high school or less, some college, college graduate); travel in the past 12 months; soil-related activities, including gardening and washing fruits or vegetables; exposure to cats (or kittens [defined as a cat <1 year old]) and cat feces; ingestion of raw or undercooked meat and poultry, of specific types of meat and poultry, and of meat and poultry that had been frozen; working with meat (ie, working with meat in a job); cross-contamination from meat; ingestion of unpasteurized cow's and goat's milk; and sources of drinking water. The term *exposure* refers to the 12 months before the date the last serum sample was obtained for *T. gondii* testing. In December 2003, an amendment was made to the study in which exposure variables related to ingestion of mollusks were added to the questionnaire because sea otters in California, which frequently eat mollusks, were found to be infected with *T. gondii*, and we thought that raw mollusks might pose a risk to humans (see Discussion). The study and its amendment were reviewed and approved by the human subjects committees at the Palo Alto Medical Foundation and the CDC.

Serological tests. For each case patient, a *Toxoplasma* se-

Table 2. Adjusted Odds Ratios (aORs) Based on 4 Multivariate Models Comparing Case and Control Patients—*Toxoplasma gondii* Case-Control Study, United States, August 2002 through May 2007

Model, associated factor	aOR (95% CLs)	AR (95% CLs), ^a %
Model 1 ^b	All persons responding to the questions in the model (<i>n</i> = 473)	
Age		
≥50 vs 18–29 years	3.21 (1.01, 10.19)	8 (0, 11)
30–49 vs 18–29 years	1.13 (0.64, 1.97)	...
Sex, male vs female	5.13 (2.10, 12.58)	12 (8, 14)
Region		
Midwest vs West	3.67 (1.53, 8.79)	43 (26, 53)
Northeast vs West	3.43 (1.66, 7.09)	...
South vs West	1.50 (0.65, 3.47)	...
Work with meat, yes vs no	3.15 (1.09, 9.10)	5 (2, 7)
Have kittens		
≥3 vs 0	27.89 (5.72, 135.86)	10 (9, 13)
1–2 vs 0	0.64 (0.26, 1.56)	...
Eat locally produced cured, dried, or smoked meat, yes vs no	1.97 (1.18, 3.28)	22 (7, 32)
Eat rare lamb, yes vs no	8.39 (3.68, 19.16)	20 (17, 21)
Eat raw ground beef, yes vs no	6.67 (2.09, 21.24)	7 (5, 8)
Microwave meat, yes vs no	0.44 (0.24, 0.81)	22 (8, 46) ^c
Drink unpasteurized goat's milk, yes vs no	5.09 (1.45, 17.80)	4 (1, 5)
Drink untreated water from a stream, lake, or pond, yes vs no	3.11 (0.92, 10.51)	...
Model 2 ^d	Persons receiving amended questionnaire (including oyster, muscle, and clam consumption) and responding to the questions in the model (<i>n</i> = 380)	
Age		
≥50 vs 18–29 years	2.50 (0.54, 11.56)	...
30–49 vs 18–29 years	0.72 (0.38, 1.38)	...
Sex, male vs female	7.30 (2.34, 22.82)	13 (8, 14)
Region		
Midwest vs West	3.32 (1.23, 8.91)	39 (13, 54)
Northeast vs West	2.67 (1.15, 6.16)	...
South vs West	1.60 (0.61, 4.20)	...
Work with meat, yes vs no	2.75 (0.83, 9.15)	...
Have kittens		
≥3 vs 0	35.36 (4.00, 312.94)	10 (8, 13)
1–2 vs 0	0.62 (0.23, 1.70)	...
Eat locally produced cured, dried, or smoked meat, yes vs no	2.10 (1.16, 3.82)	23 (7, 33)
Eat rare lamb, yes vs no	9.26 (3.79, 22.63)	20 (17, 22)
Eat raw ground beef, yes vs no	7.55 (1.75, 32.65)	7 (4, 8)
Microwave meat, yes vs no	0.42 (0.21, 0.83)	23 (7, 50) ^c
Eat any raw oysters, clams, or mussels, yes vs no	2.22 (1.07, 4.61)	16 (4, 24)
Model 3 ^e	All women responding to the questions in the model (<i>n</i> = 446)	
Age		
≥50 vs 18–29 years	3.42 (0.84, 13.96)	...
30–49 vs 18–29 years	1.14 (0.63, 2.07)	...
Region		
Midwest vs West	4.92 (1.96, 12.34)	47 (28, 57)
Northeast vs West	3.48 (1.60, 7.58)	...
South vs West	1.42 (0.56, 3.60)	...
Have kittens		
≥3 vs 0	72.74 (8.78, 602.80)	11 (10, 14)
1–2 vs 0	0.63 (0.25, 1.60)	...
Eat locally produced cured, dried, or smoked meat, yes vs no	2.48 (1.45, 4.26)	28 (15, 36)
Eat sausage, yes vs no	1.72 (0.94, 3.16)	...
Eat rare lamb, yes vs no	10.27 (4.40, 24.01)	20 (17, 21)

Table 2. (Continued.)

Model, associated factor	aOR (95% CLs)	AR (95% CLs), ^a %
Eat raw ground beef, yes vs no	5.78 (1.78, 18.71)	7 (4, 9)
Microwave meat, yes vs no	0.41 (0.22, 0.79)	24 (7, 49) ^c
Drink unpasteurized goat's milk, yes vs no	5.92 (1.59, 22.09)	4 (1, 5)
Model 4 ^f	All pregnant women responding to the questions in the model (n = 300)	
Region		
Midwest vs West	4.57 (1.29, 16.17)	58 (30, 71)
Northeast vs West	4.65 (1.58, 13.67)	...
South vs West	1.37 (0.37, 5.11)	...
Have kittens		
≥3 vs 0	76.15 (8.27, 701.12)	13 (12, 19)
1–2 vs 0	1.10 (0.37, 3.26)	...
Eat previously frozen ground pork, often/sometimes vs never	2.30 (1.12, 4.74)	22 (6, 33)
Eat rare lamb, yes vs no	5.20 (1.58, 17.09)	13 (6, 16)
Eat raw ground beef, yes vs no	5.39 (1.38, 21.01)	9 (3, 11)
Microwave meat, yes vs no	0.33 (0.12, 0.87)	26 (5, 73) ^c
Drink unpasteurized goat's milk, yes vs no	7.12 (1.56, 32.44)	6 (2, 7)
Wash hands after handling raw meat, always/sometimes vs never	0.32 (0.09, 1.10)	...

NOTE. AR, attributable risk; CLs, confidence limits.

^a Denoted for aORs significant at $P < .05$ only. Data are ARs unless otherwise indicated.

^b Summary AR, 78% (95% CLs, 69%, 83%); summary AR excluding region, sex, and age, 52% (95% CLs, 40%, 58%).

^c Prevented fraction.

^d Summary AR, 77% (95% CLs, 63%, 83%); summary AR excluding region and sex, 57% (95% CLs, 42%, 63%).

^e Summary AR, 75% (95% CLs, 64%, 81%); summary AR excluding region, 54% (95% CLs, 41%, 59%).

^f Summary AR, 79% (95% CLs, 59%, 86%); summary AR excluding region, 50% (95% CLs, 30%, 56%).

rological profile (TSP) and an avidity test were performed at the PAMF-TSL, revealing that these patients had been recently infected (acute TSP). The TSP is comprised of the Sabin-Feldman dye test (IgG) [19], double-sandwich IgM enzyme-linked immunosorbent assay (ELISA) [20], immunoglobulin A (IgA) ELISA [21], immunoglobulin E (IgE) ELISA [22], and the differential agglutination (AC/HS) test [23]. Each test was performed and results were interpreted as described elsewhere [17]. An acute TSP (ie, a Sabin-Feldman dye test titer $\geq 1:2048$, an IgM ELISA result ≥ 5.0 , an acute pattern in the differential agglutination test, and positive IgA and IgE ELISA results) correlates 100% with an acute infection acquired within 6 months in seroconversion studies of pregnant women in Europe and in serological studies of patients with lymphadenopathy in the United States [16, 17]. In addition, all case patients had a low avidity test result consistent with a recent infection [24, 25]. A nonacute TSP is never observed in patients who have been infected < 6 months [16, 17].

Statistical methods. The study data were entered into an electronic database at the PAMF-TSL and then transferred to the CDC without personal identifiers for analysis. Data analysis was performed with SAS software, version 9.1 (SAS Institute). Crude odds ratios (ORs) were calculated for all variables to ascertain the initial risk factors associated with toxoplasmosis. Variables with $P < .15$ for the associated crude OR were entered into a logistic regression model and were retained in the final

model if significant at $P < .10$. Age, sex, and region were included in all models to adjust for potential confounding. Model fit was assessed and determined to be adequate by the Hosmer-Lemeshow χ^2 test. Individual attributable risks (ARs) or prevented fractions and their 95% confidence limits (CLs) were calculated for each significant ($P < .05$) adjusted OR by standard methods [26–28]. We also provide summary ARs and summary ARs excluding significant nonmodifiable risk factors (region, age, and/or sex, depending on the model) with associated 95% CLs.

A separate model was used to evaluate raw oysters, mussels, and clams because the question pertaining to these items was added 2 years after the initiation of the study, resulting in fewer participants among which to evaluate the effect of this exposure. Separate models were also used to evaluate women and pregnant women. There was not a sufficient number of men in the study to run a separate model for them alone.

χ^2 tests were used to evaluate differences between case and control patients with respect to age, sex, race, ethnicity, and education. Continuous demographic variables were compared using pooled t tests.

RESULTS

Of 24,910 persons tested during the study period, 6119 (24.6%) were positive for IgG, and 340 (1.4%) had a low avidity test

result suggestive of a recently acquired infection [23, 24]. Of these 340 patients, 221 (65.0%) had a confirmatory serological test result consistent with an infection being acquired within 6 months of the date the serum sample was obtained. Of the 221 recently infected persons, 148 (67.0%) agreed to participate in the study and completed the survey, whereas 73 (33.0%) persons declined to participate or did not respond. The 148 case patients were similar to the 73 nonrespondents with respect to age (mean, 34.7 vs 34.4 years; $P = .79$), sex (85.1% vs 83.6% female; $P = .76$), and region (11.0% vs 15.5% for the Midwest, 39.7% vs 44.6% for the Northeast, 26.0% vs 18.9% for the South, and 23.3% vs 21.0% for the West; $P = .52$). Of the 148 case patients, 45 (30.4%) were symptomatic, and none were infected with human immunodeficiency virus. In total, 413 persons agreed to participate as control patients during the study period.

Among the 148 case patients, 76 (60.3%) of the 126 women were pregnant; among the 413 control patients (412 of whom provided information on sex), 301 (76.4%) of the 394 women were pregnant. Table 1 shows the demographic characteristics of the case patients compared with those of the control patients. Case and control patients were similar with respect to race, ethnicity, and region of the United States but differed somewhat with respect to age and sex. The time between the date of serum sampling and the date of completion of the questionnaire was approximately the same for both the case and the control patients, with 91.8% of case patients and 87.2% of control patients completing the questionnaire within 6 months of the date their serum sample was obtained.

In multivariate analysis of all persons, the following factors increased the risk of recent *T. gondii* infection (compared with the absence of the factor if the comparison group is not stated): age (≥ 50 vs 18–29 years); sex (male vs female); region (Midwest vs West and Northeast vs West); working with meat; having 3 or more kittens; eating locally produced cured, dried, or smoked meat; eating rare lamb; eating raw ground beef; and drinking unpasteurized goat's milk (model 1 in table 2). There was no evidence that the risk resulting from consumption of unpasteurized goat's milk was associated with travel outside the United States. Among those persons who had not traveled outside the United States within the past 12 months, 5 (6.1%) of 82 case patients drank unpasteurized goat's milk, compared with 4 (1.6%) of 250 control patients (OR, 3.9; 95% CIs, 1.05, 15.2); among those who had traveled outside the United States within the past 12 months, 2 (3.5%) of 57 case patients drank unpasteurized goat's milk, compared with 4 (2.8%) of 141 control patients (OR, 1.24; 95% CIs, 0.22, 6.99), indicating that the risk associated with unpasteurized goat's milk was not limited to those who had traveled outside the United States. Using a microwave to cook meat decreased the risk of recent infection. There was no effect modification in the logistic regression mod-

els. ARs or prevented fractions with 95% CIs are also shown in table 2. The model-based AR was 78% (95% CIs, 69%, 83%) for all factors combined and was 52% (95% CIs, 40%, 58%) if region of the country, age, and sex were excluded.

In a separate multivariate model that restricted data to the time period during which persons received the amended questionnaire that included questions about the consumption of oysters, clams, and muscles, the results were similar except that eating raw oysters, clams, and muscles was associated with an increased risk for recent *T. gondii* infection; however, working with meat and drinking unpasteurized goat's milk were not significant risk factors (model 2 in table 2). In the model for all women, the results were similar to those for all persons except that working with meat was not a significant risk factor (model 3 in table 2). In the model for pregnant women alone, the results were again similar to those for all persons except that age group was not significantly associated with the risk of recent *T. gondii* infection and that eating previously frozen ground pork was significantly associated with risk (model 4 in table 2).

DISCUSSION

In our case-control study, we identified eating raw oysters, clams, or mussels as a new risk factor for recent *T. gondii* infection. Oysters, clams, and mussels are filter feeders that concentrate *T. gondii*, as has been shown under experimental conditions [29]. Sea otters in California have been found to be infected with *T. gondii* [30], and it is likely that they are often infected by eating mollusks, which filter *T. gondii* from seawater. The seawater in California is thought to be contaminated by *T. gondii* oocysts that originate from cat feces, survive or bypass sewage treatment, and travel to the coast through river systems [30, 31]. *T. gondii* has been identified in a California mussel by polymerase chain reaction and DNA sequencing [32]. In light of the potential presence of *T. gondii*, pregnant women and immunosuppressed persons should be aware of this potential risk associated with eating raw oysters, muscles, and clams.

In addition to this new factor, we identified a number of risk factors that have been previously associated with *T. gondii* infection, such as eating undercooked meat and drinking unpasteurized goat's milk [15, 18, 33]. Drinking untreated water also elevated the risk, although not significantly so. We were not able to find an overall estimate of consumption of unpasteurized goat's milk in the United States. However, as noted in Results only 1.6% of the control patients indicated that they drank unpasteurized goat's milk in the past 12 months, so consumption of unpasteurized goat's milk must be relatively uncommon. In the United States, meat has been found to be contaminated with *T. gondii*, especially pork, lamb, and wild-game meat (such as venison) [33]. Eating frozen ground pork

was associated with an increased risk of recent *T. gondii* infection in pregnant women. Adequate freezing (-12°C or lower for at least 24 h) will inactivate *T. gondii* cysts, but cysts have remained viable for >11 days at -6.7°C [34]. Hill et al [35] found that US frozen-meat display-case temperatures are not even as cold as -6.7°C . Working with meat was also associated with recent *T. gondii* infection in our study. It is possible that some persons who work with meat could inadvertently ingest undercooked meat (for example, if gloves were not worn or were removed incorrectly). It is also possible that cooks who work with meat could taste dishes before the meat was fully cooked or could inadvertently ingest undercooked meat from their hands. A national survey of pregnant women found that many women were not aware of the risk of toxoplasmosis associated with undercooked meat [36]. It is important that pregnant women and immunosuppressed persons not eat undercooked meat and follow safe-cooking guidelines [15, 37]. In addition, unpasteurized goat's milk and untreated water should be avoided.

Microwave cooking of meat was associated with a reduced risk of recent *T. gondii* infection (table 2). This may seem counterintuitive, because microwave cooking does not always heat meat evenly [38]. However, in the United States microwave cooking is often associated with reheating already-cooked meat or with defrosting or cooking frozen meat (for example, TV dinners). Because deep freezing (-12°C or lower) usually kills *T. gondii* cysts in meat [34, 35], people who frequently eat frozen meat cooked in a microwave oven may have a lower risk of infection with *T. gondii*. As noted above, however, if meat is not sufficiently frozen, *T. gondii* cysts can remain viable.

It is interesting that the increased risk associated with exposure to kittens was limited to respondents who had 3 or more kittens. This suggests that a litter of kittens may be responsible for the risk. Kittens that have access to rodents and birds often become infected with *T. gondii* when they are weaned, grow older, and develop hunting skills. Via their feces, a litter of kittens could contaminate the environment with millions of oocysts, which could survive for months or years in the soil [39]. To avoid environmental contamination, a litter box should be used and feces cleaned up daily, preferably by a nonpregnant, nonimmunosuppressed person [15, 37]. Daily cleaning of the litter box helps prevent *T. gondii* infection because the organism requires 1 or more days to sporulate and become infectious after being shed in the feces [15]. Although the questionnaire inquired about many soil-related risk factors (eg, gardening, washing fruits, and vegetables), none significantly increased the risk of *T. gondii* infection in multivariate analysis.

We found an AR of 78% for all the factors in the multivariate model (model 1 in table 2) and of 52% when excluding the effect of age, sex, and region, which are not modifiable risk

factors. In other words, we were not able to explain the risk for 48% of the infections in our study (excluding the non-modifiable risk factors). This is consistent with the findings of a multicenter study in Europe that was not able to explain 14%–49% of the risk for *T. gondii* infection, depending on the center [18]. Sometimes people may be unaware of their exposures or may have difficulty recalling specific risks that occurred [40].

One strength of our study is that case patients had serologically defined recent *T. gondii* infection, so their recent behavior and other risk factors correspond to the time of their infection. One limitation of our study is that the case and control patients were not precisely matched by age; however, age is not necessarily related to the risk of recent infection (rather than prevalent infection). In addition, we controlled for age in the multivariate analysis. There was a higher proportion of men among the case patients than the control patients (14.9% vs 4.3%); however, this was a result of the volunteer nature of the study and was controlled for in the multivariate analysis. We also presented subset analyses for women and pregnant women. Although this study was conducted at the only *Toxoplasma* reference laboratory in the United States, our findings may not be generalizable to the entire US population. It is possible that both case and control patients who mentioned risk factors to their clinicians were more likely to be referred for testing and agree to the survey. It should be noted that ARs depend on the prevalence of exposure to the specific risk factor (and strength of association) in the population. Accordingly, the ARs presented apply only to the study population and may be different for other groups.

Toxoplasma gondii is a widely prevalent parasite that is potentially responsible for significant morbidity and mortality in the congenitally infected child and those with immunosuppression and for high morbidity in all persons in the form of ocular disease. In the present study, we have determined the risk factors among US patients referred for testing. Reduction of *T. gondii* contamination of meat and increased education of health professionals and the public about the factors identified in this study could help to further reduce the burden of toxoplasmosis in the United States.

Acknowledgments

We thank Drs Julie Parsonnet, Monica Parise, and John Williamson for their critical review of the manuscript and helpful comments.

Financial support. Centers for Disease Control and Prevention.

Potential conflicts of interest. J.G.M. is the director of and J.S.R. is the founder of and a consultant for the Palo Alto Medical Foundation Toxoplasma Serology Laboratory. However, this is a public health, prevention, and risk factor study and should not represent a specific conflict of interest. The Palo Alto Medical Foundation is a nonprofit organization and maintains the only *Toxoplasma* reference laboratory in the United States. All other authors: no conflicts.

References

1. Alford CA Jr, Stagno S, Reynolds DW. Congenital toxoplasmosis: clinical, laboratory, and therapeutic considerations, with special reference to subclinical disease. *Bull N Y Acad Med* **1974**; 50:160–81.
2. Kimball AC, Kean BH, Fuchs S. Congenital toxoplasmosis: a prospective study of 4,048 obstetric patients. *Am J Obstet Gynecol* **1971**; 111: 211–18.
3. Guerina NG, Hsu H-W, Meissner H, et al. Neonatal serologic screening and early treatment for congenital *Toxoplasma gondii* infection. *N Engl J Med* **1994**; 330:1858–63.
4. Holland GM. Ocular toxoplasmosis: a global reassessment. I. Epidemiology and course of disease. *Am J Ophthalmol* **2003**; 136:973–88.
5. Jones JL, Kruszon-Moran D, Wilson M, McQuillan G, Navin T, McAuley JB. *Toxoplasma gondii* infection in the United States: seroprevalence and risk factors. *Am J Epidemiol* **2001**; 154:357–65.
6. Smith RE, Ganley JP. Ophthalmic survey of a community. I. Abnormalities of the ocular fundus. *Am J Ophthalmol* **1972**; 74:1126–30.
7. Jones JL, Kruszon-Moran D, Sanders-Lewis K, Wilson M. *Toxoplasma gondii* infection in the United States, 1999–2004, decline from the prior decade. *Am J Trop Med Hyg* **2007**; 77:405–10.
8. Montoya JG, Remington JS. Management of *Toxoplasma gondii* infection during pregnancy. *Clin Infect Dis* **2008**; 47:554–66.
9. Tan HK, Schmidt D, Stanford M, et al. Risk of visual impairment in children with congenital toxoplasmic retinochoroiditis. *Am J Ophthalmol* **2007**; 144:648–53.
10. Kodjikian L, Wallon M, Fleury J, et al. Ocular manifestations in congenital toxoplasmosis. *Graefes Arch Clin Exp Ophthalmol* **2006**; 244: 14–21.
11. Wallon M, Kodjikian L, Binquet C, et al. Long-term ocular prognosis in 327 children with congenital toxoplasmosis. *Pediatrics* **2004**; 113: 1567–72.
12. Kieffer F, Wallon M, Garcia P, Thulliez P, Peyron F, Franck J. Risk factors for retinochoroiditis during the first 2 years of life in infants with treated congenital toxoplasmosis. *Pediatr Infect Dis J* **2008**; 27: 27–32.
13. Phan L, Kasza K, Jalbrzikowski J, et al. Longitudinal study of new eye lesions in treated congenital toxoplasmosis. *Toxoplasmosis Study Group. Ophthalmology* **2008**; 115:553–9.
14. Gras L, Wallon M, Pollak A, et al. Association between prenatal treatment and clinical manifestations of congenital toxoplasmosis in infancy: a cohort study in 13 European centres. *Acta Paediatrica* **2005**; 94:1721–31.
15. Lopez A, Dietz V, Wilson M, Navin TR, Jones JL. Preventing congenital toxoplasmosis. Centers for Disease Control and Prevention. *MMWR Recomm Rep* **2000**; 49(RR-2):59–68.
16. Montoya JG. Laboratory diagnosis of *Toxoplasma gondii* infection and toxoplasmosis. *J Infect Dis* **2002**; 185(Suppl 1):S73–82.
17. Montoya JG, Remington JS. Studies on the serodiagnosis of toxoplasmic lymphadenitis. *Clin Infect Dis* **1995**; 20:781–9.
18. Cook AJ, Gilbert RE, Buffolano W. Sources of *Toxoplasma* infection in pregnant women: European multicentre case-control study. European Research Network on Congenital Toxoplasmosis. *BMJ* **2000**; 321: 142–7.
19. Sabin AB, Feldman HA. Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoan parasite (*Toxoplasma*). *Science* **1948**; 108:660–3.
20. Naot Y, Remington JS. An enzyme-linked immunosorbent assay for detection of IgM antibodies to *Toxoplasma gondii*: use for diagnosis of acute acquired toxoplasmosis. *J Infect Dis* **1980**; 142:757–66.
21. Stepick-Biek P, Thulliez P, Araujo FG, Remington JS. IgA antibodies for diagnosis of acute congenital and acquired toxoplasmosis. *J Infect Dis* **1990**; 162:270–3.
22. Wong SY, Hajdu MP, Ramirez R, Thulliez P, McLeod R, Remington JS. Role of specific immunoglobulin E in diagnosis of acute toxoplasma infection and toxoplasmosis. *J Clin Microbiol* **1993**; 31:2952–9.
23. Dannemann BR, Vaughan WC, Thulliez P, Remington JS. Differential agglutination test for diagnosis of recently acquired infection with *Toxoplasma gondii*. *J Clin Microbiol* **1990**; 28:1928–33.
24. Hedman K, Lappalainen M, Seppää I, Mäkelä O. Recent primary *Toxoplasma* infection indicated by a low avidity of specific IgG. *J Infect Dis* **1989**; 159:736–40.
25. Montoya JG, Huffman HB, Remington JS. Evaluation of the immunoglobulin G avidity test for diagnosis of toxoplasmic lymphadenopathy. *J Clin Microbiol* **2004**; 42:4627–31.
26. J.J. Schlesselman. Case-control studies. Oxford: Oxford University Press, **1982**:221.
27. Benichou J. A review of adjusted estimators of attributable risk. *Stat Methods Med Res* **2001**; 10:195.
28. Efron B, Tibshirani R. An introduction to the bootstrap. New York: Chapman & Hall, **1993**.
29. Lindsay DS, Collins MV, Mitchell SM, et al. Survival of *Toxoplasma gondii* oocysts in eastern oysters (*Crassostrea virginica*). *J Parasitol* **2004**; 90:1054–7.
30. Conrad PA, Miller MA, Kreuder C, et al. Transmission of *Toxoplasma*: clues from the study of sea otters and sentinels of *Toxoplasma gondii* flow into the marine environment. *Int J Parasitol* **2005**; 35:1155–68.
31. Miller MA, Gardner IA, Kreuder C, et al. Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*). *Int J Parasitol* **2002**; 32:997–1006.
32. Miller MA, Miller WA, Conrad PA, et al. Type X *Toxoplasma gondii* in a wild mussel and terrestrial carnivores from coastal California: new linkages between terrestrial mammals, runoff and toxoplasmosis of sea otters. *Int J Parasitol* **2008**; 38:1319–28.
33. Dubey JP, Jones JL. *Toxoplasma gondii* infection in humans and animals in the United States. *Int J Parasitol* **2008**; 38:1257–78.
34. Kotula AW, Dubey JP, Sharar AK, Andrews CD, Shen SK, Lindsay DS. Effect of freezing on infectivity of *Toxoplasma gondii* tissue cysts in pork. *J Food Prot* **1991**; 54:687–90.
35. Hill DE, Benedetto SMC, Coss C, McCary JL, Fournet VM, Dubey JP. Effects of time and temperature on the viability of *Toxoplasma gondii* tissue cysts in enhanced pork loin. *J Food Prot* **2006**; 69:1961–5.
36. Jones JL, Ogunmodede F, Scheffel J, et al. Toxoplasmosis-related knowledge and practices among pregnant women in the United States. *Infect Dis Obstet Gynecol* **2003**; 11:139–45.
37. Centers for Disease Control and Prevention. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. *MMWR Recomm Rep* **2009**; 58(No. RR-4):1–207.
38. Lunden A, Arvid U. Infectivity of *Toxoplasma gondii* in mutton following curing, smoking, freezing or microwave cooking. *Int J Food Microbiol* **1992**; 15:357–63.
39. Frenkel JK, Ruiz A, Chinchilla M. Soil survival of *Toxoplasma* oocysts in Kansas and Costa Rica. *Am J Trop Med Hyg* **1975**; 24:439–43.
40. Boyer KM, Holfels E, Roizen N, et al. Risk factors for *Toxoplasma gondii* infection in mothers of infants with congenital toxoplasmosis: implications for prenatal management and screening. *Toxoplasmosis Study Group. Am J Obstet Gynecol* **2005**; 192:564–71.