**Toxoplasma gondii** Seropositivity and Co-Infection with TORCH Pathogens in High-Risk Patients from Qatar

Marawan A. Abu-Madi, Jerzy M. Behnke, and Haydee A. Dabritz*

College of Arts and Sciences, Department of Health Sciences, Qatar University, Doha, Qatar; School of Biology, University of Nottingham, Nottingham, United Kingdom; Infant Botulism Treatment and Prevention Program, California Department of Public Health, Richmond, California

Abstract. Testing of patients who are deemed to be at high risk for TORCH pathogens, e.g., pregnant women, their fetuses, neonates, and acquired immunodeficiency syndrome (AIDS) patients, is important so that specific treatment can be initiated. This study included 1,857 such patients between 2005 and 2008. Logistic regression was used to evaluate factors associated with *Toxoplasma gondii* seropositivity. Among 823 women of childbearing age, 35.1% and 5.2% tested positive for *T. gondii* IgG and IgM, respectively. Three infants ≤ 6 months of age (0.8% of 353) were congenitally infected. Factors associated with *T. gondii* IgG seropositivity included older age, East Mediterranean or African nationality, positive cytomegalovirus (CMV) and herpes simplex virus (HSV)-1 serostatus, and negative rubella IgG results. The decreasing prevalence of IgM antibodies between 2005 and 2008 suggested that exposure to *T. gondii* from food or environmental sources declined over this period in Qatar. Population-based studies of newborns would be helpful to accurately estimate incidence of congenital toxoplasmosis.

INTRODUCTION

*Toxoplasma gondii* is a ubiquitous parasite whose definitive hosts are members of the Felidae (cat family). Cats shed millions of environmentally resistant oocysts in their feces after primary infection and are usually without clinical manifestations of disease.1–4 Intermediate hosts include almost all warm-blooded mammals and birds, including humans, who accumulate infectious, quiescent stages (bradyzoites) of the parasite in their tissues, particularly in skeletal muscle and the brain.5,6 Intermediate hosts may acquire infection by consuming raw or undercooked flesh from other intermediate hosts,5 or by ingesting oocysts from the environment.7–10 Environmental sources of *T. gondii* (oocysts) include soil, water, shellfish, fruits, and vegetables.8–10

*Toxoplasma gondii* is of particular concern in humans because of the potential for transmitting the disease to the unborn fetus if the mother is infected for the first time during pregnancy.11–13 Toxoplasmosis most commonly manifests as a mild, flu-like illness with low-grade fever, myalgia, malaise, and headache, but primary infection in humans may also cause spontaneous abortion, fetal mental and psychomotor retardation, retinchoroiditis, encephalitis, and hepatitis.6,12–14 Patients with a history of recent miscarriage, ocular infection, jaundice, hepatosplenomegaly, and cirrhosis of the liver may be referred into a testing protocol termed “TORCH” (*T. gondii*, other [if done, e.g., syphilis, varicella zoster virus, human immunodeficiency virus, and parvovirus B19], rubella, cytomegalovirus [CMV], and herpes simplex viruses [HSVs]), to rule out infections with similar clinical presentations.

The TORCH infections can cause serious illness or death to the fetus or neonate, so TORCH testing is important to protect the health of neonates that may have been exposed to one or more TORCH pathogens in utero. It is also important to identify the etiologic agent associated with clinical disease in symptomatic adults so that appropriate treatment can be initi-ated. The classic triad of symptoms associated with congenitally acquired toxoplasmosis includes hydrocephaly, intracranial calcifications, and ocular lesions.15–17 The fetus causes birth defects and blindness, hearing loss, and mental retardation.18 Congenital CMV has similar manifestations to toxoplasmosis and rubella that include sensorineural hearing loss, mental retardation, and retinchoroiditis.19 Neonatal HSV (usually acquired during vaginal delivery) may lead to external infection of the skin, eyes, and mouth, central nervous system (CNS) infection (encephalitis), or disseminated infection involving several organs such as the brain, liver, and lung.20 Disseminated infection is a more frequent cause of mortality.21 Most clinical disease in neonates is caused by HSV type 2 (HSV-2) infection.21,22

In Qatar, better prenatal care and greater vigilance with regard to TORCH infections may lead to earlier and more effective therapy. No specific *T. gondii* prevention program exists in Qatar other than routine prenatal counseling, which may or may not include advice about how to prevent toxoplasmosis. Increasing concern has been raised about toxoplasmosis because of the large, indigenous feral cat population in the capital city of Doha, where most of the inhabitants live. Doha and its surrounding communities had experienced problems controlling rodents for decades, so domestic cats were introduced in the 1960s to ameliorate the problem. The cat population is now estimated to exceed 2 million animals (Cat Control Unit, personal communication). Cats are rarely kept as pets in Qatar and the vast majority leads a feral existence on the streets, congregating near human dwellings, businesses, restaurants, and in the market places where food for human consumption is prepared and traded.23 They survive by scavenging on garbage and preying on rodents, and some are supplemented with food by the local residents.

This analysis updates and extends an earlier study of patients referred for TORCH testing in Qatar.24 The aims were to determine the prevalence of *T. gondii* in the subpopulations of women of childbearing age and infants from among all patients referred for TORCH testing in Qatar, and to determine if specific TORCH pathogens (CMV, HSV-1, HSV-2, and rubella) were associated with *T. gondii* infection in patients referred for TORCH testing, after controlling for demographic *T. gondii* risk factors.

* Address correspondence to Haydee A. Dabritz, Infant Botulism Treatment and Prevention Program, California Department of Public Health, 850 Marina Bay Parkway, E-361, Richmond, CA 94804. E-mail: haydee.dabritz@cdph.ca.gov

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MATERIALS AND METHODS

Study location, selection of subjects, and inclusion criteria. Doha, the capital city of Qatar, is located on the Arabian Gulf and encompasses about 285 km². The city is populated by about 1 million residents, many of whom are immigrants from other Middle Eastern countries, Africa, and Asia. The climate of Qatar is arid, with sparse annual rainfall averaging only 0.1–3.2 cm.

The entire patient population included persons who had symptoms compatible with those of TORCH pathogens (ocular disease, hepatosplenomegaly, cirrhosis), women with a history of miscarriage(s), and their most recent child, who was usually <1 year of age. Testing was carried out in Qatari hospitals and outpatient clinics between 2005 and 2008, and patients came from such specialties as maternity, pediatrics, internal medicine, and gastroenterology. Patient confidentiality was maintained throughout and the data set were de-identified so as to mask patient identity from the investigators. The study was approved by the Medical Research Center & Research Committee at Hamad Medical Cooperation, Qatar (research protocol no. 8036/08).

Blood collection and serological tests. Each subject had 5 mL of whole blood collected by venipuncture in plain tubes. Blood samples were then transported to the virology laboratory at Hamad Medical Corporation according to hospital arrangements, centrifuged to remove blood cells, and stored at +4°C for 48 hours or frozen at −20°C for longer storage. Serologic tests for anti- T. gondii IgG and IgM antibodies were performed as previously described.24 Commercially available enzyme immunoassay Enzygnost kits (Dade Behring GmbH, Marburg, Germany) were used to detect the presence of antibodies against T. gondii, rubella virus, and CMV, and the Novagnost EIA kits (NovaTec Immunagnostika GmbH, Dietzenbach, Germany) for HSV-1/HSV-2. Sera with values of <10 IU/mL were defined as negative for rubella IgG antibodies and those with values ≥ 10 IU/mL as positive. The latter test has a published sensitivity (Se) of 100% and specificity (Sp) 98.5%. Rubella IgM testing was not included in this analysis because the testing protocol for rubella IgM included only patients with negative rubella IgG results and symptoms of fever and rash, which increases the likelihood of the patient being a clinical rubella case. For CMV, sera with values <0.5 IU/mL were defined as negative, those between 0.5 and 0.7 IU/mL as equivocal, and those >0.7 IU/mL as positive. The reported Se and Sp of this test was 99.3% and 98.2%, respectively. For CMV IgM, sera were considered negative if the ratio of the optical densities, OD sample /OD cutoff were <90%, equivocal between 90% and 100% and positive if >100%, and the manufacturer reported Se and Sp of 95% and 100%, respectively. In HSV-1 and HSV-2 Novagnost tests, sera were considered negative for HSV-1 or HSV-2 IgM or IgG antibodies if values were <8.5 IU/mL, equivocal between 8.5 and 11.5 IU/mL, and positive if >11.5 IU/mL. Se and Sp, respectively, for each of the four tests were reported as follows: HSV-1 IgG and IgM >95% and >95%, HSV-2 IgG 87.5% and 94.1%, and HSV-2 IgM >95% and >95%. The HSV-1 and HSV-2 immunoassays are considered to be semi-quantitative.

Definition of variables. Age was classified into ranges by yr as previously described,24 except that infants were classified into ages of ≤6 mo and 7–<12 mo. In this analysis, the age group of 7–<12 mo was used as the referent group because about one-quarter of infants ≤6 mo of age had IgG antibodies against T. gondii in their sera that were most likely acquired in utero. Maternally acquired antibodies usually disappear by 6 mo of age.25 However, IgG antibodies in infant sera can also appear as a result of congenital infection.26 For patients ≥12 mo of age, the categories in yr were 1–< 2, 2–10, 11–20, 21–29, 30–45, and >45. Presence of IgM antibodies in infant sera suggests that toxoplasmosis was transmitted congenitally,27,28 but the tests lack sensitivity, missing 50–75% of incident cases.27,29,30 Prevalence of T. gondii antibodies in the female population was also assessed separately by age in yr to determine T. gondii prevalence in females of childbearing age (15–45 yr).

Subjects in the study came from 55 countries that were grouped into the following geographic regions: Arabian Peninsula (Bahrain, Iraq, Kuwait, Oman, Qatar, Saudi Arabia, United Arab Emirates, and Yemen); Africa (Algeria, Egypt, Eritrea, Ethiopia, Ivory Coast, Kenya, Libya, Mauritania, Morocco, Senegal, Somalia, Sudan, and Tunisia); Asia (Afghanistan, Bangladesh, Iran, India, Indonesia, Macao, Malaysia, Nepal, North Korea, Pakistan, Philippines, Singapore, South Korea, Sri Lanka, and Vietnam); Eastern Mediterranean/Eastern Europe (Armenia, Bulgaria, Jordan, Lebanon, Palestine, Romania, Syria, and Turkey); American continent (Canada, Cuba, United States, and Venezuela); and Other/Unknown (Australia, France, New Zealand, Spain, Russia, Ukraine, and United Kingdom). Year of testing was included as a categorical variable with 2005 as the referent year. Association of T. gondii seropositivity with TORCH pathogens included rubella (IgG) status, CMV IgG/IgM status, HSV-1 IgG/IgM status, and HSV-2 IgG/IgM status.

Statistical analyses. Estimates for the incidence of congenital toxoplasmosis were determined using two methods: defining a case as an infant ≤6 mo of age with concurrent IgM and IgG T. gondii antibodies in serum; or a woman of childbearing age with IgM antibodies to T. gondii in her serum. Both methods used the total number of live births in Qatar between 2005 and 2008 as the denominator for population size and assumed that all cases of congenital toxoplasmosis or incident cases of toxoplasmosis in pregnancy would have been detected. For the estimate based on women of childbearing age with T. gondii IgM antibodies, the probability of T. gondii transmission to the fetus was assumed to be between 25% and 50%. The lower limit of the 95% confidence interval (CI) for the estimate based on 25% transmission probability and the upper limit of the 95% CI for the estimate based on 50% transmission probability delimited the ranges for the estimates derived for IgM-positive women of childbearing age. Confidence intervals were calculated using Szkllo and Nieto, Appendix A.25 The binary logistic regression analyses were conducted in Minitab Release 14.2 (Minitab Inc., State College, PA). Each explanatory factor in turn was fitted alone (one-way analyses) with the dependent variable (presence/absence of antibodies to T. gondii) and then full factorial models were simplified by using stepwise backward elimination. Factors were then fitted in minimum sufficient models where P values were considered significant if ≤0.05. The Hosmer-Lemeshow test was used to verify model fit. The Cochran-Armitage test was used to evaluate a trend of increasing T. gondii IgG seroprevalence with age and for decreasing IgM seroprevalence from 2005 to 2008 using SAS version 9.2 (SAS Institute Inc., Cary, NC).
RESULTS

Of the 1,857 patients referred for TORCH testing in 2005–2008, 4.1% (N = 3 missing data) and 30.8% of patients tested positive for IgM and IgG *Toxoplasma gondii* antibodies, respectively (Table 1). *Toxoplasma gondii* IgG and IgM seroprevalence by age group for residents of Qatar is depicted in Figure 1. Among 823 women of childbearing age (15–45 yr), 289 (35.1%) had IgG antibodies and 43 (5.2%) had IgM antibodies against *T. gondii* (N = 1 of unknown IgM status). *Toxoplasma gondii* IgG seroprevalence increased with advancing age in both males and females, with a combined prevalence of 8% in 2 to 10 years of age compared with 54% in patients over 45 years of age. The Cochran-Armitage trend test by age category showed a significant association with increasing age (Z-statistic 7.97, 7 degrees of freedom [df], P < 0.01). This was markedly different from the age prevalence of rubella virus and CMV. Rubella IgG seroprevalence ranged from 50% to 78% in all age groups 1–<2 yr and up (Table 1). In Qatar, vaccination against rubella has been mandatory for children before their first birthday since the 1970s. The CMV IgG seroprevalence was around 80% at young ages (2 to 10 years of age) and generally >90% in all age groups from 11–20 yr and up. In contrast, HSV-1 and HSV-2 IgG seroprevalence almost doubled in the age group encompassing teens (11–20 yr) compared with 2 to 10 years of age, and remained relatively stable thereafter. Notably, prevalence of HSV-1 IgG was higher than that of HSV-2 in adults (21 and up): 65–79% compared with 17–30%, respectively. Of the 353 infants in the study aged ≤6 mo, 81 (22.9%) had detectable anti-*T. gondii* IgG antibodies that may be passively

<table>
<thead>
<tr>
<th>Age group</th>
<th>Toxoplasma gondii IgG n (%)</th>
<th><em>T. gondii</em> IgM n (%)</th>
<th>Rubella IgG n (%)</th>
<th>CMV IgG n (%)</th>
<th>CMV IgM n (%)</th>
<th>HSV-1 IgG n (%)</th>
<th>HSV-1 IgM n (%)</th>
<th>HSV-2 IgG n (%)</th>
<th>HSV-2 IgM n (%)</th>
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<td>Females</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>≤6 mo (N = 167)</td>
<td>41 (25)</td>
<td>3 (2)</td>
<td>104 (62)</td>
<td>157 (95)</td>
<td>9 (5)</td>
<td>86 (52)</td>
<td>0 (0)</td>
<td>51 (31)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>7–11 mo (N = 22)</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>3 (14)</td>
<td>16 (73)</td>
<td>1 (5)</td>
<td>3 (14)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>1–&lt;2 yr (N = 16)</td>
<td>1 (6)</td>
<td>1 (6)</td>
<td>8 (50)</td>
<td>10 (62)</td>
<td>1 (6)</td>
<td>3 (19)</td>
<td>0 (0)</td>
<td>1 (6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2–10 yr (N = 38)</td>
<td>4 (11)</td>
<td>0 (0)</td>
<td>25 (66)</td>
<td>30 (79)</td>
<td>3 (8)</td>
<td>13 (34)</td>
<td>1 (3)</td>
<td>5 (13)</td>
<td>5 (13)</td>
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<tr>
<td>11–20 yr (N = 66)</td>
<td>9 (14)</td>
<td>2 (3)</td>
<td>42 (64)</td>
<td>60 (91)</td>
<td>2 (3)</td>
<td>40 (61)</td>
<td>1 (2)</td>
<td>14 (21)</td>
<td>6 (9)</td>
</tr>
<tr>
<td>21–29 yr (N = 367)</td>
<td>114 (31)</td>
<td>20 (5)</td>
<td>285 (78)</td>
<td>352 (96)</td>
<td>11 (3)</td>
<td>246 (67)</td>
<td>6 (2)</td>
<td>87 (24)</td>
<td>27 (7)</td>
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<tr>
<td>30–45 yr (N = 404)</td>
<td>167 (41)</td>
<td>21 (5)</td>
<td>300 (74)</td>
<td>394 (98)</td>
<td>10 (2)</td>
<td>301 (75)</td>
<td>2 (0.5)</td>
<td>122 (30)</td>
<td>33 (8)</td>
</tr>
<tr>
<td>&gt;45 yr (N = 76)</td>
<td>41 (54)</td>
<td>2 (3)</td>
<td>49 (64)</td>
<td>74 (97)</td>
<td>2 (3)</td>
<td>60 (79)</td>
<td>0 (0)</td>
<td>14 (18)</td>
<td>4 (5)</td>
</tr>
<tr>
<td>Total (N = 1,156)</td>
<td>378 (33)</td>
<td>49 (4)</td>
<td>816 (71)</td>
<td>1093 (95)</td>
<td>39 (3)</td>
<td>752 (65)</td>
<td>10 (1)</td>
<td>294 (25)</td>
<td>76 (7)</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>≤6 mo (N = 186)</td>
<td>40 (22)</td>
<td>0 (0)</td>
<td>127 (68)</td>
<td>178 (96)</td>
<td>8 (4)</td>
<td>101 (54)</td>
<td>1 (1)</td>
<td>60 (32)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>7–11 mo (N = 26)</td>
<td>1 (4)</td>
<td>0 (0)</td>
<td>2 (8)</td>
<td>17 (65)</td>
<td>1 (4)</td>
<td>5 (19)</td>
<td>0 (0)</td>
<td>3 (12)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>1–&lt;2 yr (N = 19)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>11 (58)</td>
<td>16 (84)</td>
<td>2 (11)</td>
<td>3 (16)</td>
<td>2 (11)</td>
<td>3 (16)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2–10 yr (N = 35)</td>
<td>2 (6)</td>
<td>1 (3)</td>
<td>18 (51)</td>
<td>28 (80)</td>
<td>1 (3)</td>
<td>10 (29)</td>
<td>0 (0)</td>
<td>3 (9)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>11–20 yr (N = 35)</td>
<td>6 (17)</td>
<td>1 (3)</td>
<td>20 (57)</td>
<td>31 (89)</td>
<td>0 (0)</td>
<td>22 (63)</td>
<td>0 (0)</td>
<td>6 (17)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>21–29 yr (N = 123)</td>
<td>37 (30)</td>
<td>7 (6)</td>
<td>84 (68)</td>
<td>116 (94)</td>
<td>6 (5)</td>
<td>81 (66)</td>
<td>2 (2)</td>
<td>32 (26)</td>
<td>7 (6)</td>
</tr>
<tr>
<td>30–45 yr (N = 163)</td>
<td>62 (38)</td>
<td>12 (7)</td>
<td>115 (71)</td>
<td>158 (97)</td>
<td>3 (2)</td>
<td>108 (66)</td>
<td>1 (0.6)</td>
<td>37 (23)</td>
<td>11 (7)</td>
</tr>
<tr>
<td>&gt;45 yr (N = 114)</td>
<td>46 (40)</td>
<td>6 (5)</td>
<td>74 (63)</td>
<td>111 (97)</td>
<td>5 (4)</td>
<td>75 (66)</td>
<td>0 (0)</td>
<td>19 (17)</td>
<td>7 (6)</td>
</tr>
<tr>
<td>Total (N = 701)</td>
<td>194 (28)</td>
<td>27 (4)</td>
<td>451 (64)</td>
<td>655 (93)</td>
<td>26 (4)</td>
<td>405 (58)</td>
<td>6 (1)</td>
<td>163 (23)</td>
<td>29 (4)</td>
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</table>

*CMV = cytomegalovirus; HSV = herpes simplex virus.

Figure 1. *Toxoplasma gondii* IgG and IgM seroprevalence by age category and gender in high-risk patients referred for TORCH testing in Qatar between 2005 and 2008. Exact binomial 95% confidence intervals (CIs) were computed using SAS software (SAS Institute Inc.).
TORCH PATHOGENS IN HIGH-RISK PATIENTS FROM QATAR

transferred by the placenta, compared with only 3 (0.8%) with IgM antibodies (Table 1). All three infants with IgM titers also tested positive for IgG antibodies and were most likely congenitally infected. Using cases as defined for infants and total live births of 13,401 in 2005, 14,120 in 2006, 15,681 in 2007, and 17,210 in 2008, the annual incidence of congenital toxoplasmosis from 2005 to 2008 was estimated to be 0.5 cases (95% CI 0.1–1.5 cases) per 10,000 live births. If the number of cases was based on the detection of anti- T. gondii IgM antibodies in high-risk women of childbearing age with the parameters defined in the methods section, the estimated incidence of congenital toxoplasmosis between 2005 and 2008 ranged from 0.9–5.5 cases per 10,000 live births.

In the one-way analyses, presence of T. gondii IgG antibodies was positively associated with older age; African, Asian, or East Mediterranean/European nationality; positive CMV IgG status; positive HSV-1 IgG status; and positive HSV-2 IgM status (Table 2). It was negatively associated with male gender and rubella IgG seropositivity. In contrast, there was no association between T. gondii IgM status and age or nationality, but positive tests were associated with seropositivity to HSV-1 IgG and HSV-2 IgM antibodies. In addition, the one-way analysis revealed a lower prevalence of positive T. gondii IgM results in 2008 compared with 2005 (Table 2). A subsequent trend test indicated a decline in the prevalence of anti- T. gondii IgM antibodies between 2005 and 2008 (Z-statistic 2.09, 1 df, $P = 0.037$).

In multifactorial analyses (Table 3), anti- T. gondii IgG seropositivity was associated with older age ($\chi^2 78.43, 7$ df, $P < 0.001$); African, East Mediterranean, or East European Table 2

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>IgM positive* (%)</th>
<th>Odds ratio (95% confidence interval)</th>
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<tr>
<td>Sex</td>
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<tr>
<td>Female</td>
<td>49 (4)</td>
<td>1.00</td>
</tr>
<tr>
<td>Male</td>
<td>27 (4)</td>
<td>0.91 (0.56–1.46)</td>
</tr>
<tr>
<td>Age group</td>
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<tr>
<td>≤ 6 mo</td>
<td>3 (1)</td>
<td>Undefined</td>
</tr>
<tr>
<td>7–12 mo</td>
<td>0 (0)</td>
<td>Referent</td>
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<td>1–2 yr</td>
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<tr>
<td>2–10 yr</td>
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<tr>
<td>11–20 yr</td>
<td>3 (3)</td>
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</tr>
<tr>
<td>21–29 yr</td>
<td>27 (6)</td>
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<tr>
<td>30–45 yr</td>
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<tr>
<td>&gt; 45 yr</td>
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<td>African</td>
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<td>1.53 (0.20–12.0)</td>
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<tr>
<td>2006</td>
<td>17 (4)</td>
<td>0.78 (0.42–1.45)</td>
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<td>2007</td>
<td>19 (4)</td>
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<td>2008</td>
<td>13 (3)</td>
<td>0.50 (0.25–0.98)‡</td>
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<td>CMV-IgG seropositivity</td>
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</tr>
<tr>
<td>HSV-1 IgG seropositivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>20 (3)</td>
<td>1.00</td>
</tr>
<tr>
<td>Yes</td>
<td>56 (5)</td>
<td>1.73 (1.03–2.90)‡</td>
</tr>
<tr>
<td>HSV-1 IgM seropositivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>76 (4)</td>
<td>1.00</td>
</tr>
<tr>
<td>Yes</td>
<td>0 (0)</td>
<td>Undefined</td>
</tr>
<tr>
<td>HSV-2 IgG seropositivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>62 (4)</td>
<td>1.00</td>
</tr>
<tr>
<td>Yes</td>
<td>14 (3)</td>
<td>0.68 (0.38–1.23)</td>
</tr>
<tr>
<td>HSV-2 IgM seropositivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>65 (4)</td>
<td>1.00</td>
</tr>
<tr>
<td>Yes</td>
<td>11 (11)</td>
<td>3.03 (1.55–5.94)§</td>
</tr>
</tbody>
</table>

*Three patients missing IgM results were excluded.
†Global $P$ value.
‡$P < 0.05$
§$P < 0.01$
¶$0.05 < P < 0.10$
|| Defined in Materials and Methods.
against infection. Such precautions include cooking meat, especially lamb and pork, until it is well done; thorough washing of cutting boards used to prepare meat; wearing gloves when gardening; rigorous hand washing after handling raw meat or working in the soil; and avoiding contact with cat feces. Pregnant women who must clean out cat litter boxes should wear disposable gloves and collect feces daily, because it takes about 24 hr for oocysts to sporulate and become infectious at room temperature. Forty-three women of childbearing age (5.2%) tested positive for anti- \textit{T. gondii} IgM antibody. If they were pregnant, IgG avidity testing is the preferred method to confirm recent infection, because IgM antibodies can persist for months after initial infection in some individuals. Alternate testing algorithms use higher IgM serologic titers as good indicators of acute infection when economic considerations preempt additional tests.

The presence of IgM antibody in three infants ≤ 6 mo of age suggested that they were congenitally infected. However, IgM testing reportedly fails to detect 50–75% of infected infants; therefore, 6–12 cases of congenital toxoplasmosis may have occurred between 2005 and 2008. The results of IgM testing in women of childbearing age with high-risk pregnancies (history of miscarriage) support this hypothesis, because 43 cases of toxoplasmosis acquired during pregnancy would be expected to result in 11–21 cases of congenital toxoplasmosis, assuming a 25–50% probability of transmission to the fetus \textit{in utero}. In north Jordan, women with a history of 3 to 7 miscarriages were twice as likely to test seropositive for \textit{T. gondii} as women with normal pregnancies. Women without high-risk pregnancies are also at risk for acquiring toxoplasmosis and transmitting it to their fetus, but we do not know if the risk is similar to or differs from that of women with high-risk pregnancies in Qatar. Our estimates for the incidence of congenital toxoplasmosis in Qatar are therefore speculative and should be confirmed by population-based studies. In the absence of universal screening, primary prevention of congenital toxoplasmosis through heightened maternal education efforts can be an effective strategy to prevent transmission to the fetus.

The overall \textit{T. gondii} IgG seroprevalence (33%) during the time period (Table 1) was similar to cross-sectional studies from Bahrain (28%), Saudi Arabia (32%, 25%, 36%, and 29% reported in 1991, 2001, 2002, and 2006, respectively), and the United Arab Emirates (34%), but much lower than that reported in two studies from Kuwait (96% and 58%) in the 1980s. Overall, prevalence of \textit{T. gondii} IgM antibodies (4%) was also similar to countries in the Arabian Peninsula, e.g., Saudi Arabia (5% and 5.6% in the eastern region and Makkah, respectively), and the United Arab Emirates (3%). Comparisons between the present and earlier studies should be made with caution, because different tests were used and most of the studies were conducted in specific subpopulations, such as pregnant women or women of childbearing age. Toxoplasma gondii IgM prevalence in high-risk patients declined significantly between 2005 and 2008, but this was not the case for IgG seroprevalence. The latter is a measure of individuals infected months or years earlier; therefore, it is unlikely we would detect a decline until incidence decreases significantly for 5 to 10 years. Declining IgM seroprevalence suggests that knowledge about how to prevent \textit{T. gondii} infection may have improved, the prevalence of \textit{T. gondii} cysts in food animals in the region is declining, or that the feral cat control program is reducing the quantity of oocysts entering the environment. According to the Ministry of Public Health,
there have been no changes in public education programs with
regard to *T. gondii*. The prevalence of *T. gondii* in meat, most
likely sheep and goats, imported into Qatar for human con-
sumption is unknown. Reports from other countries in the
region since 2001 found *T. gondii* prevalence of 23% to 52% in
sheep, suggesting that lamb, if it is undercooked (a prevalent cultural practice in this area), could be a source of
*T. gondii* infection. Since the feral cat control program began
in 2006, 9,637 cats have been spayed or neutered and 282 cats
of 871 tested (32.3%) were serologically positive for *T. gondii*
antibodies. It seems likely that the cat control program may be
reducing environmental exposure to *T. gondii* oocysts by con-
trolling reproduction and lessening the number of susceptible
kittens entering the population, but testing of residents living
within and outside trapping areas would be required to verify
this hypothesis.

Multifactorial analysis found that the presence of *T. gondii*
IgG antibodies was positively related to age, African or East
Mediterranean/East European nationality, and the presence of
CMV IgG and HSV-1 IgG antibodies, whereas it was negatively
associated with rubella IgG seropositivity. Increasing *T. gondii*
seroprevalence with age has been well documented, suggest-
ing that exposure is constant over the life span. Persons
originating from African nations, the East Mediterranean, and
Eastern Europe were 2.3 times more likely to have *T. gon-
dii* IgG antibodies than persons who came from countries in
the Arabian Peninsula. The increased risk may reflect cultural
differences in eating practices, such as consumption of rare
or undercooked meat and choice of meat, and weather pat-
terns that promote oocyst survival. Udoms and others also
found that *T. gondii* seroprevalence in Mediterranean Arabs
was about twice as high as that of persons from the Arabian
Gulf countries or the United Arab Emirates. Consumption of
rare lamb or goat is common in the Eastern Mediterranean,
Turkey, and Iran. Extremely high temperatures and pro-
longed periods without rainfall in the Arabian Peninsula may
inactivate oocysts in the environment rapidly, thereby reducing
transmission to humans directly by soil contact or indirectly by
food animals such as sheep and goats. For example, oocysts can
survive 32 d at 35°C but only 9 d at 40°C. Minimum and max-
imum temperatures in Qatar range from 25 to 45°C, respec-
tively, from June to September and rarely dip below 10°C in
the winter months, and it is unclear how long oocysts might
survive in this desert climate. In Costa Rica, oocysts in soil
survived for up to 1 yr even when air temperatures reached
30°C. Studies of oocyst survival in the native Qatari climate
would help to determine if oocysts represent a health hazard
to persons with soil contact or if specific seasons of the year
pose a greater hazard to humans and animals for acquiring
*T. gondii* from the environment.

Three other TORCH pathogens were associated with
*T. gondii* IgG seropositivity in the present analysis. Adjusting
for age and nationality, *T. gondii*-seropositive patients were
1.94 and 1.35 times more likely than seronegative patients
to have previous exposure to CMV and HSV-1, respectively
(Table 3). They were about half as likely as seronegative
patients to have IgG antibodies to rubella virus (odds ratio
[OR] = 0.59). The CMV is transmitted directly from person
to person, particularly between children, in saliva, urine, and
genital secretions. Most of the children and adolescents in
Qatar appear to have been exposed to CMV with seropreva-
lences of 79% in the 2–10-year and 91% in the 11–20-year age
groups. Poor socioeconomic conditions that are characterized
by overcrowding and a lack of hand hygiene, and placing chil-
dren in daycare facilities, promote CMV transmission. Low
socioeconomic status is also associated with *T. gondii* infec-
tion, which may explain why persons with positive CMV
serostatus were more likely to be seropositive for *T. gondii*
IgG antibodies. The HSV-1 is an alpha-herpes virus that pro-
duces orolabial blisters or lesions. It remains latent for the life
of the infected host and causes intermittent viral shedding, at
which time it is infectious to susceptible persons. In the past,
most primary HSV-1 infections were oral, and were acquired
in childhood by direct mucosal or cutaneous contact with an infected person. Improved hand hygiene in devel-
oped countries has reduced the incidence of oral HSV-1 in
childhood, whereas primary genital HSV-1 (acquired during
sexual contact) has become more common in the teen years
and young adulthood. In Israel, genital HSV-1 has become
more prevalent than HSV-2. The association of HSV-1 IgG
antibodies with *T. gondii* IgG seropositivity may be related
to poor socioeconomic conditions during childhood, because
HSV-1 IgG was highly prevalent in the 2–10-year (34%) and
11–20-year (61%) age groups. Much like the association with
CMV, an association between HSV-1 and socioeconomic fac-
tors has been documented in other countries.

Unfortunately, vaccines for CMV and HSV-1 have not yet
been developed, but a vaccine against rubella virus has been
available since 1969. Most nations in the Middle East, includ-
ing Qatar, have rubella in their national immunization sched-
ules, whereas almost all nations in Africa do not. Because the
rubella virus IgG antibody test does not discriminate between
vaccine-induced and naturally acquired immunity, seropos-
itivity to rubella IgG is likely to be a surrogate for vaccine status.

The reverse association for rubella virus with *T. gondii* IgG
seropositivity is probably related to higher socioeconomic sta-
tus, which entails better access to health care, more hygienic
living conditions, and less occupational contact with soil.
Soil contact, either by occupational exposure or gardening,
is a well-recognized risk for *T. gondii* seropositivity in many
nations. It would have been desirable to obtain infor-
mation about soil exposure and other factors known to be
associated with *T. gondii* seropositivity, such as cat contact and
consumption of undercooked meat, from patients referred for
TORCH testing, but these data were not collected. Such data
could help determine the extent to which environmental ex-
sposure to oocysts and/or exposure by the food chain contribute
to human toxoplasmosis in Qatar, so that appropriate preven-
tive measures can be adopted.

Only limited conclusions can be drawn about the preva-
ience of *T. gondii* in Qatar from this study, because it focused
on a specific, high-risk population. If patients included in the
study were more likely to be infected with toxoplasmosis, we
would then overestimate the prevalence of *T. gondii* in Qatar.
Conversely, if we failed to detect asymptomatic cases, which
are more likely, the burden of toxoplasmosis may be underesti-
ated. Nonetheless, the proportion of previously infected per-
sons was similar to other countries in the Arabian Peninsula.
The high percentage of at-risk females in their childbearing
years (65%) suggests that pregnant women should receive coun-
seling during their antenatal visits on how to prevent *T. gon-
dii* infection. Population-based studies or universal screening
of newborns over a specified time period could elucidate the
true burden of congenital toxoplasmosis in Qatar and suggest
priorities for public health action. Future studies would benefit from questions that assess exposure to previously identified risk factors for T. gondii infection, such as recreational or occupational exposure to soil, drinking water source, contact with domestic pets and food animals, and practices related to cooking and preparing meat.

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Authors’ addresses: Marawan A. Abu-Madi, College of Arts and Sciences, Department of Health Sciences, Qatar University, Doha, Qatar, E-mail: abumadi@qu.edu.qa. Jerzy M. Behnke, School of Biology, University of Nottingham, University Park, Nottingham, UK, E-mail: jerzy.behnke@nottingham.ac.uk. Haydee A. Dabritz, Infant Botulism Treatment and Prevention Program, California Department of Public Health, Richmond, CA, E-mail: haydee.dabritz@cdph.ca.gov.

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