

Prevalence and associated risk factors of *Toxoplasma gondii* in female farmworkers of southeastern Turkey

Nebiye Yentur Doni¹, Zeynep Simsek², Gulcan Gurses¹, Fadile Yildiz Zeyrek³, Canan Demir²

¹ Department of Medical Microbiology, Vocational School of Health Services, Harran University, Sanliurfa, Turkey

² Department of Public Health, Faculty of Medicine, Harran University, Sanliurfa, Turkey

³ Department of Medical Microbiology, Faculty of Medicine, Harran University, Sanliurfa, Turkey

Abstract

Introduction: There are a limited number of studies of the agricultural population about the prevalence of *Toxoplasma gondii* (*T. gondii*) infection throughout the world. The aim of this study was to determine the prevalence and risk factors of toxoplasmosis in female farmworkers in the southeastern region of Turkey.

Methodology: The optimum sample size was determined using the Epi Info Program, and blood samples were collected from 684 women of reproductive age. Serum samples were analyzed by qualitative and quantitative methods for anti-*T. gondii* IgG and IgM antibodies with a commercially available enzyme immunoassay. Questionnaires through which socio-demographic information was collected were applied during face-to-face interviews of subjects who gave their consent to participate in the study. Crude odds ratios (ORs) and 95% confidence intervals (CIs) were calculated, and Chi-square analyses and logistic regression were performed.

Results: Of the 684 women, the prevalence of toxoplasmosis was determined to be 58.3% and 1% for IgG and IgM, respectively. Employment as a seasonal farmworker, increasing age, and having had three or more pregnancies were found to be the crucial associated risk factors that affect the prevalence of *T. gondii* infection ($p < 0.05$). In contrast to these, the provincial development level was found to be a confounding factor. There was no association between toxoplasmosis and household size, education attainment, and poverty in the bivariate analyses.

Conclusions: The findings show that control and serological screening programs should be implemented nationwide in Turkey for the reliable and fast detection of congenital *Toxoplasma* infections.

Key words: Toxoplasmosis; agricultural population; reproductive-aged women; IgG; IgM.

J Infect Dev Ctries 2015; 9(1):087-093. doi:10.3855/jidc.5824

(Received 28 August 2014 – Accepted 12 November 2014)

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Introduction

Toxoplasmosis is one of the more prevalent parasitic zoonoses worldwide, caused by *Toxoplasma gondii* (*T. gondii*). The seroprevalence of *T. gondii* varies in geographical regions within a country and within different population groups based on different environmental, social, and cultural lifestyle factors [1]. It has been reported that toxoplasmosis is present in every country; the seropositivity rates range from less than 10% to over 90% [2], and the seroprevalence of *Toxoplasma* infection widely differs, between 30% and 60%, in both developed and developing countries [3]. According to data obtained from the World Health Organization (WHO), more than 60% of the newly identified infectious agents that have affected people over the past few decades have been caused by pathogens originating from animals or animal products

[4]. Toxoplasmosis is mainly acquired by eating raw or undercooked meat that contains *T. gondii* tissue cysts, by ingesting oocysts shed by cats from soil (contact with soil, gardening, farming, and handling/eating unwashed vegetables and fruits), by drinking contaminated water, and by vertical transmission from mother to fetus through the placenta during pregnancy [5-7]. Maternal exposure to toxoplasmosis during the first trimester of pregnancy may cause mental retardation, epilepsy, death [7], low birth weight, blindness, hearing loss, development of mental and motor disabilities during childhood [6,8], abortion, hydrocephalus, neurological disorders, and retinochoroiditis [9]. Therefore, women of reproductive age are an important risk group in view of primary and secondary toxoplasmosis control programs.

The Southeastern Anatolia Project (GAP) region is situated in a semiarid plain at 550 meters; it is a massive dam and waterways project currently under construction in the Southeastern Anatolia Region (SAR) of Turkey. The large-scale irrigation projects that are parts of the SAR give way to the transformation of the climate to warm and humid in southeastern Anatolia. Worldwide, the prevalence of *Toxoplasma* is high in humid tropical areas and low in hot and dry areas. Prevalence of the parasite in arctic areas is also low [10,11].

Working in a soil-related occupation was defined by the longest-held job and included farmworkers, farm operators, and farm managers [7]. Contact with soil and gardening have been determined to be risk factors for *T. gondii* infection [12]. To our knowledge, the prevalence of *T. gondii* infection in female farmworkers of childbearing age in the GAP region has not been previously reported. In light of these data, we performed a cross-sectional study to determine the prevalence of *T. gondii* infection in female farmworkers living in the GAP region and the risk factors associated with the seropositivity of *T. gondii*.

Methodology

Study area

This study was conducted between January and April of 2013 in nine provinces of the SAR (Adıyaman, Batman, Diyarbakır, Gaziantep, Kilis, Mardin, Siirt, Şanlıurfa, and Şırnak) of Turkey. According to the 2012 census records, the total population of the SAR was approximately 7,816,173 (nearly one-tenth of the total Turkish population), where 25% of the population was employed in agriculture and 49.5% of the agriculture workers were women [13]. The GAP region is the one of the least developed regions of Turkey.

Study population

In this study, *T. gondii* variables from the representative multipurpose cross-sectional research data of the SAR 2013 Agricultural Health Survey, conducted by the authors in collaboration with the Harran University Scientific Research Council and SAR Regional Development Administration, were analyzed. This survey was approved by the ethics committee of the Faculty of Medicine at Harran University. The National Turkish Statistics Institution's data were used to calculate the optimum sample size using a 95% confidence level, and the selected households were chosen using the probability proportional to size method. The cluster size was

identified as 10 households working in agriculture. If there was more than one woman of reproductive age, one woman was randomly enrolled in the study using the person selection method. Employment in agriculture and animal husbandry was determined in 1,077 households. A total of 345 women (32.0%) were rejected from enrollment into the study or refused to give blood sample, 41 of whom (3.6%) could not be located during home visits, and there was no appropriate person to interview in seven (0.6%) households. Therefore, this study was successfully conducted in 684 households.

During home visits, the aim of the study was explained to the participants, and informed consent was obtained from each participant. Data were collected during a face-to-face interview using a structured questionnaire that included socio-demographic information on family size, age, education level, type of work (seasonal migratory worker/family farmer), provincial development level, number of pregnancies (parity), knowledge of *T. gondii*, poverty, employment in husbandry, consumption of raw meat, fruits or vegetables, and washing of hands, fruits and vegetables.

Blood samples and serologic markers

Blood samples were collected in gel vacuum tubes from female farmworkers between 15 and 49 years of age. The tubes containing the blood were then labeled and transferred to the Harran University Microbiology Laboratory under suitable conditions, and centrifuged and stored at -80°C until use. Serum samples were analyzed by a quantitative method for *T. gondii* IgG antibodies and by a qualitative method for *T. gondii* IgM antibodies with the commercially available chemiluminescent microparticle immunoassays (Abbott Architect, Abbott Laboratories, Wiesbaden, Germany). *T. gondii* IgG antibody concentration levels were expressed as international units IU/mL, and a result equal to or greater than 3 IU/mL was considered positive for *T. gondii* and indicated past or acute infection. *T. gondii* IgG antibody concentration levels < 1.6 IU/mL were considered negative. The unit for *T. gondii* IgM results was index or sample to cutoff (S/CO). The results of IgM antibodies with ≥ 0.60 index (≥ 1.00 S/CO) were considered positive for *T. gondii* and indicated a recently acquired infection. The results of IgM antibodies with < 0.50 index (< 0.83 S/CO) were considered negative for *T. gondii*. Next, all IgG and IgM positive serum samples were tested for IgG avidity. Low IgG avidity was defined as < 50% avidity, high IgG avidity was defined as > 60%

avidity, and equivocal IgG avidity was defined as $50 \leq \text{Avi}\% \leq 60$ by using IgG avidity assay. A high avidity index ($> 60\%$ avidity) for IgG antibodies was considered to be a strong indication that an infection took place more than four months before.

Statistical analysis

Data entry and analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 11.5. Associations among the variables were assessed using crude odds ratios (ORs), and 95% confidence intervals (CIs) were calculated, and Chi-square analyses were performed. The level of statistical significance was accepted as $p \leq 0.05$ for each statistically significant factor.

Results

A total of 684 female farmworkers of reproductive age were enrolled in this study. The mean age of the participants was 36.2 ± 9.8 years. The mean household size was 8.1 ± 3.5 persons. Of the participants, approximately 54.4 % were > 35 years of age, 69% were illiterate, 34.9% were seasonal migratory farmworkers, 58.4% had five or more children, 61.2% gave birth without a healthcare professional present, 90.7% lived in poverty, 85% had no terrain toilets in the agricultural area, 71.9% used unsafe water (e.g., spring water, river water, boreholes, well water, groundwater, and storage water in containers) and had no hand washing mechanism, including soap and water, in the fields. The families raised livestock such as cattle, sheep, poultry, and bees, at rates of 55%, 26%, 32%, and 1%, respectively. One out of every three farmworker families consumed the meat of their infected animals.

Of the 684 female farmworkers of reproductive age, 399 (58.3%) had anti-*T. gondii* IgG antibodies, and seven (1.1%) were also seropositive for *T. gondii* IgM antibodies. Five (0.73%) were seropositive for both IgM and IgG antibodies. Two (0.29%) of them were positive for IgM alone. Five positive samples for IgG and IgM antibodies to *T. gondii* had high avidity antibodies, suggesting prior infection.

As seen in Table 1, there was no significant association between *T. gondii* infection and household size, education attainment, and poverty ($p > 0.05$). On the other hand, a significant association was found with the type of work (OR = 2.0; 95% CI = 1.1–3.8). *T. gondii* infection was 1.5 times higher among seasonal female farmworkers than female family farmworkers (95% CI: 1.2–1.9). *T. gondii* infection was significantly ($p < 0.05$) higher in women > 35

years of age, and in those who had had three or more pregnancies.

As shown in Table 2, the provincial development level was a confounding factor, while working as a seasonal farmworker, having had three or more pregnancies, and increasing age were found as the only major risk factors affecting the prevalence of *T. gondii*.

Discussion

This study emphasizes that agricultural female farmworkers are a disadvantaged group prone to contagious diseases; 58.3% and 1.1% of the agricultural female workers of reproductive age included in this study were positive for IgG and IgM antibodies to *T. gondii*, respectively. To our knowledge, this study is the first to report the prevalence on representative agricultural women of reproductive age and to estimate the at-risk group in agricultural communities. In last decade, studies that included pregnant women and women of reproductive age who were admitted to hospitals in different parts of Turkey reported that the seroprevalence of *T. gondii* ranged between 28% and 69.6% [14-18]. Compared to other studies conducted in different provinces of Turkey, the seroprevalence rate we found was similar to that of a study conducted in Sanliurfa, a province of the southeastern region of Turkey [14], but higher than that found in other studies on pregnant women admitted to hospitals [15-18]. The major contributing factors to such a high prevalence was attributed to working in agriculture, poverty, and exposure to unhealthy conditions, contaminated soil through gardening, and contaminated water and vegetables. The high seroprevalence might also be a consequence of the GAP, which causes the geographical climate to change to warm and humid in the study area. As the average winter temperature and humidity increase, the effective sporulation of *T. gondii* oocysts also rises, as has been reported previously [11].

T. gondii exposure has been attributed to low socioeconomic status [19]. The majority of the female farmworkers in our study lived in poverty and were exposed to many risk factors for *T. gondii* infection due to poverty; however, poverty was not associated with *T. gondii* infection in our study. The effect of poverty on toxoplasmosis was complex and could be attributed to many factors, such as an unhygienic environment and unsafe water for drinking, washing vegetables, and kitchen utensils.

Table 1. Risk factors for *T. gondii*

Variables	Total		Positive		Negative	
	n	%	n	%	n	%
Age group						
15–24 years	84	12.3	29	7.3	55	19.3
25–34 years	229	33.5	126	31.6	103	36.1
35–45 years	371	54.2	244	61.2	127	44.6
χ^2 ; P; OR (CI 95%)	29.063; 0.000					
Household size						
Six and below	240	35.1	154	64.2	86	35.8
Seven and above	444	64.9	245	55.2	199	44.8
χ^2 ; P; OR (CI 95%)	0.358; 0.55; 1.0 (0.7–2.2)					
Education attainment						
Illiterate	472	69.0	273	57.8	199	42.2
Primary school and above	212	31.0	126	59.4	86	40.6
χ^2 ; P; OR (CI 95%)	0.153; 0.696; 1.0 (0.8–1.3)					
Type of work						
Family farmers	445	65.1	235	52.8	210	47.2
Seasonal migratory workers	239	34.9	164	68.6	75	31.4
χ^2 ; P; OR (CI 95%)	15.991; 0.001; 1.5 (1.2–1.9)					
Provincial development level						
Underdeveloped	519	75.9	290	55.9	229	44.1
Developed	165	24.1	109	66.1	56	33.9
χ^2 ; P; OR (CI 95%)	5.43; 0.001; 1.3 (1.1–1.6)					
Number of pregnancies (parity)						
Two or fewer	118	17.3	49	41.5	69	58.5
Three or more	566	82.7	350	61.8	216	38.2
χ^2 ; P; OR (CI 95%)	16.57; 0.000; 2.3 (1.5–3.4)					
Poverty						
Yes	620	90.6	362	58.4	258	41.6
No	64	9.4	37	57.8	27	42.2
χ^2 ; P; OR (CI 95%)	0.008; 0.515; 0.977 (0.580–1.645)					
Total	684	100.00	399	58.3	285	41.7

Table 2. Summary result of logistic regression

Independent variables	B	SE	Wald	P	EXP (B)	CI 95%
Type of work (Seasonal female farmworkers)	0.589	0.172	11.714	0.001	1.80	1.3–2.5
Developmental level (Underdeveloped area)	0.361	0.192	3.531	0.060	0.70	0.5–1.02
Women’s ages (35 and older)	0.418	0.173	5.840	0.016	1.52	1.08–2.13
Number of pregnancies (Three or more)	0.581	0.225	6.666	0.010	1.79	1.15–2.78
Constant	-1.510	0.575	6.900	0.009	0.221	

B: coefficient for the constant (also called the intercept) in the null model; SE: standard errors associated with the coefficients; Wald: Wald chi-square value and 2-tailed p-value used in testing the null hypothesis that the coefficient (parameter) is 0; EXP(B): odds ratio for an independent variable; CI 95%: confidence interval of odds ratio

The female farmworkers in this study used this unsafe water for drinking and for washing vegetables, fruits, and kitchen utensils. Drinking and using this unsafe water contaminated with oocysts shed by cats might be a risk factor for toxoplasmosis. The female farmworkers might be exposed to toxoplasmosis by contact with human and animal urine or feces that were found in every open part of the agricultural area due to the absence of toilets.

Consumption of raw fruits and vegetables contaminated with *T. gondii* oocysts might be also a risk factor for toxoplasmosis. Raw meatball consumption is common in GAP, and raw meat has been reported to be a high risk for *T. gondii* infection by direct ingestion of tissue cysts [14]. Female farmworkers eat meat occasionally because they can not afford to buy it due to poverty. One out of every three farmworker families consumed the infected meat of their animals. Consumption of infected animal meat might be a threat for toxoplasmosis transmission; it has been reported that tissue cysts are mostly detected in the intercostal, leg, and diaphragmatic muscles of sheep and poultry, which are frequently consumed by humans and have been implicated as an important source of toxoplasmosis [20,21]

In this study, we found that increasing age was associated with the seropositivity of *T. gondii*, which was reported previously in other studies [22-27]. This might be explained by longer exposure to risk factors and by the transmission methods of *T. gondii*.

Employment as a seasonal migratory farmworker was the second major risk factor for *T. gondii* infection. Seasonal migratory female farmworkers were approximately 1.5 times more likely to have *T. gondii* infection than were family farmworkers. This might be due to the women working in agricultural labor out of their homes, living in shelters in under poor sanitation conditions, and having low hygiene practices.

Having had three or more pregnancies was found to be the third major risk factor for *T. gondii* infection in our study. The risk of *T. gondii* infection presented as 2.3 times higher among female farmworkers who had had three or more pregnancies than among women with two or fewer pregnancies. This might derive from unawareness of the disease, and the fact that 61.2% of the female farmworkers gave a birth without a healthcare professional present in unhealthy, unsanitary conditions, and used delivery devices contaminated with soil. This finding showed that the local midwife can change this situation by focusing on infectious diseases during the antenatal care screening

program. The similarity of our study's finding to that of other studies emphasizes the significant correlation between toxoplasmosis cases and the number of pregnancies in women [5,28]. In light of this data, consultancy services should be offered to female farmworkers of reproductive age to prevent, control, avoid, and bring awareness to *T. gondii* infection, congenital toxoplasmosis, and the risk factors involved. Female farmworkers of reproductive age should be encouraged not to be afraid of congenital toxoplasmosis, but they should be trained about the risk factors and prevention of diseases through an antenatal care program for women of reproductive age. All of these routes might cause a change in the attitudes of the women.

In our study, educational attainment was not associated with *T. gondii* infection, as it had previously been reported [27]. This result might be as a consequence of the distribution of the female farmworkers' education levels. In our study, the majority of female farmworkers (69%) were illiterate, and the others had attended only primary school or above. Approximately all of the female farmworkers had no knowledge about the *T. gondii* infection. Thus, there was no difference between these two education levels. We noticed that primary school was not enough for changing attitudes about preventing, controlling, and gaining awareness of disease as a source of infection. On the other hand, *T. gondii* seroprevalence might be reduced with a high academic education level, as reported in other works [7,23]. In our study, 64.9% of the female farmworkers' families had at least seven members, and 55.2% of these were infected with *T. gondii*. The majority of the families were very crowded, and 58.4% of the female farmworkers had five or more children. However, household size was not associated with exposure to *T. gondii* infection

Conclusions

These data suggest that approximately one out of every two female farmworkers in the SAR were infected with *T. gondii*, while the remainder were healthy, and thus susceptible to acute toxoplasmosis during their reproductive period, putting their infants at risk for congenital toxoplasmosis. Therefore, it is crucial that female farmworkers of reproductive age be trained about the transmission and control routes of toxoplasmosis and prevention of soil-related risks of toxoplasmosis. Healthy living areas should be implemented in agricultural areas for farmworkers, and healthy nutritional attitudes should be adopted by farmworkers. Agricultural workers at high risk for

toxoplasmosis who have limitations in accessing healthcare services should be routinely monitored [29].

Based on our knowledge, control and serological screening programs should be implemented in the SAR in Turkey for the control of congenital *T. gondii* infections, as they have been implemented in Australia and France [30]. Reliable and faster diagnostic tests should be developed for both mother and fetus.

Acknowledgements

The authors thank the Harran University Scientific Council and Southeast Anatolian Regional Development Administration for supporting this research, and all of the female farmworkers for participating.

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Corresponding author

Nebiye Yentur Doni

Department of Medical Microbiology, Vocational School of Health Services, Harran University, İpekyol Avenue, No:1, Haliliye Sanliurfa/Turkey Şanlıurfa, Turkey

Phone: +90 414 313 30 00

Fax: +90 414 318 32 11

Email: n_doni@hotmail.com

Conflict of interests: No conflict of interests is declared.