



Seroprevalence and Associated Risk Factors of *Toxoplasma gondii* Among Pregnant Women in Southwest Iran

Shahrzad Soltani ¹, Ali Dalir Ghaffari ², Mehdi Sagha Kahvaz ¹, Mohamad Sabaghan ³, Marzieh Pashmforosh ^{3,*} and Masoud Foroutan ^{4,**}

¹USERN Office, Abadan University of Medical Sciences, Abadan, Iran

²Department of Parasitology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

³Behbahan Faculty of Medical Sciences, Behbahan, Iran

⁴Research Center for Environmental Contaminants (RCEC), Abadan University of Medical Sciences, Abadan, Iran

*Corresponding author: Behbahan Faculty of Medical Sciences, Behbahan, Iran. Email: marzie_pf@yahoo.com

**Corresponding author: Research Center for Environmental Contaminants (RCEC), Abadan University of Medical Sciences, Abadan, Iran. Email: masoud_foroutan_rad@yahoo.com

Received 2021 May 19; Revised 2021 October 16; Accepted 2021 November 02.

Abstract

Background: Acute *Toxoplasma gondii* infection during pregnancy period can cause congenital toxoplasmosis. The aim of this study was to assess the seroprevalence rate of immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies against *T. gondii* infection during pregnancy and the associated risk factors in southwest Iran.

Methods: This study was performed on 88 pregnant women from October to December 2019 in Khorramshahr County, Khuzestan province, Iran. Anti-*T. gondii* IgG and IgM antibodies were tested through enzyme-linked immunosorbent assay (ELISA) method.

Results: Following serological assays, 38.63% (34/88) and 2.27% (2/88) serum samples were positive for IgG and IgM antibodies, respectively. Also, a statistically significant association was observed between IgG seroprevalence and drinking of unpurified water ($P = 0.015$).

Conclusions: The serological evidence revealed that pregnant women of southwest Iran had moderate exposure to *T. gondii* parasite. Since the risk of acquiring acute toxoplasmosis in pregnant women is clinically important, we highly recommend regular screening tests for *T. gondii* infection during pregnancy period.

Keywords: *Toxoplasma*, Enzyme-linked Immunosorbent Assay, Serology, Pregnancy, Iran

1. Background

Toxoplasmosis, caused by a well-known intracellular parasite known as *Toxoplasma gondii*, is considered as one of the main infectious agents during pregnancy (1-4). Cats (Family: Felidae) serve as definitive host (1). The infection is chiefly transmitted through consumption of raw/unwashed vegetables or fruits, ingestion of raw/undercooked meat contaminated with tissue cysts, drinking water contaminated with mature oocysts, vertically from mother to the fetus, and scarcely by blood transfusion and organ transplantation (5-8).

In general, in immunocompetent persons, *T. gondii* infection is predominantly asymptomatic, while in immunocompromised individuals can cause life-threatening outcomes with poor prognosis (6, 9). Besides, seronegative pregnant mothers are other risk groups for acquiring *T. gondii* infection (5, 10). Over one-third of the human population are latently seropositive for the parasite worldwide

(6, 10, 11). For instance, according to a review study by Pappas et al., the seroprevalence rate of *T. gondii* infection among pregnant women and those of childbearing age was reported 25.3-75.2%, 6.1-77.5%, 0.8-63.9%, and 8.2-63.2% in the Africa, Americas, Asia, and Europe continents, respectively (12). Acute *T. gondii* infection during pregnancy is oftentimes asymptomatic or may manifest as flu-like signs with fatigue, mild fever, and/or lymphadenopathy. Without prenatal screening programs in a specific region, acute toxoplasmosis in pregnant women will remain overlooked and left untreated (5, 13).

In numerous recent studies, the seroepidemiology of *T. gondii* during pregnancy period was estimated in different provinces of Iran, including 41.8% in Golestan (14), 62.6% in Mazandaran (15), 43.3% in Isfahan (16), 43.5% in Qom (17), 33.5% in Hamadan (18), 37.8% in Zanjan (19), 34.1% and 21.5% in Khuzestan (20, 21), and 14% and 30.8% in Sistan and Baluchistan (22, 23).

2. Objectives

Since there is lack of epidemiological data regarding seroprevalence rate of *T. gondii* infection amongst pregnant women in Khorramshahr county, this study was conducted to evaluate the seroprevalence rate of this parasite during pregnancy and its associated risk factors in this region.

3. Methods

3.1. Study Population

This cross-sectional study was performed on 88 pregnant women from Khorramshahr county, Khuzestan province (Southwest Iran). The participants were selected from patients referred to Valiasr hospital from October to December 2019. Following receiving the approval from the Ethical Committee (IR.BHN.REC.1399.007), all pregnant women voluntarily consented to be tested for the specific antibodies (immunoglobulin G (IgG)/immunoglobulin M (IgM)) of *T. gondii* infection. The inclusion criteria were pregnant women aged 13 - 45 years; *T. gondii* infection confirmed by both clinical (by an expert physician) and laboratory (positive pregnancy tests) tests, and giving a written informed consent.

3.2. Serology

In order to detect specific IgG and IgM antibodies against *T. gondii* infection, 5 mL of venous blood was gathered from each subject. As previously described (20, 24), the blood samples were centrifuged at 4000 rpm for five minutes and then stored at -20°C until tested. The IgG and IgM antibodies were detected via a commercially enzyme-linked immunosorbent assay (ELISA) kit based on the manufacturer's instructions.

3.3. Questionnaire

Each subject filled out a structured questionnaire containing demographic information, including age range, source of drinking water, place of residence, consumption of raw/undercooked meat, education level, contact with cat, reason for referral to hospital, and history of abortion. This questionnaire was developed and accomplished, as earlier described (20, 24, 25).

3.4. Statistical Analysis

All data were imported into the statistical package for the social sciences (SPSS) software (version 21) (SPSS Inc., Chicago, IL, USA) for more analysis. For statistical analysis, we used the Pearson chi-square and Fisher's exact tests. The P-value less than 0.05 was defined as the significance level.

4. Results

4.1. Participants

In this study, 88 pregnant women were tested for antibodies to *T. gondii* and filled out a questionnaire from October to December 2019. In terms of residence, 59.10% (52/88) of the subjects were from urban regions, and 40.90% (36/88) were from rural areas. In terms of education, 77.27% (68/88) had a high school diploma or lower degree, and 22.73% (20/88) had a university degree (Table 1).

4.2. Seroprevalence of Anti-Toxoplasma gondii Antibodies

The overall seroprevalence of *T. gondii* infection in pregnant women was 40.90% (36/88). Briefly, 38.63% (34/88) and 2.27% (2/88) of serum samples were detected positive for IgG and IgM antibodies, respectively. No positive sample was found for both IgG and IgM.

4.3. Risk Factors

Among the main risk factors associated with toxoplasmosis, a statistically significant association was found between the seroprevalence of anti-*T. gondii* IgG and source of drinking water (consumption of unpurified water). The other risk factors showed no significant association (Table 1).

5. Discussion

Acute toxoplasmosis in pregnant women can cause congenital toxoplasmosis. The infection may result in severe and progressive disease with high morbidity and mortality in fetuses and newborns (2, 3, 5). Based on published reports, congenital toxoplasmosis is responsible for approximately 1.2 million disability-adjusted life years (DALYs) annually (5, 13). According to Torgerson & Mastroiacovo (2013) (13), approximately 190,100 cases (179,300-206,300) with congenital toxoplasmosis happen each year worldwide. Moreover, the authors declared that South America along with some Middle Eastern and low-income countries, had the highest burden for congenital toxoplasmosis (13). In two separate meta-analysis papers with a global perspective, the seroprevalence rate of latent and acute toxoplasmosis during pregnancy was calculated as 33.8% and 1.1%, respectively (5, 10). In a meta-analysis published by Foroutan-Rad et al. (26), the pooled seroprevalence rate of *T. gondii* infection among Iranian pregnant women from 1990 to 2015 was reviewed. Based on the results, IgG and IgM antibodies were estimated at 38% and 4%, respectively, and the pooled seroprevalence was 41%. Furthermore, the seropositivity for *T. gondii* infection ranged from 26.6% to 77.2%, with more endemicity in

Table 1. Demographic Characteristics and Risk Factors Related to IgG (Latent) and IgM (Acute) Seroprevalence of *Toxoplasma gondii* in Pregnant Women in Khorramshahr County, Khuzestan Province, Iran During Oct-Dec 2019^{a, b}

Characteristic	Number Tested (Percent of Total Tested)	IgG Positive	P-Value	IgM Positive	P-Value	Total
Total	88 (100)	34 (38.63)		2 (2.27)		36 (40.90)
Age (y)			0.642		0.079	
10 - 20	17 (19.32)	7 (41.17)		0 (0)		7 (41.17)
21 - 30	42 (47.73)	19 (45.23)		1 (2.38)		20 (47.61)
31 - 40	27 (30.68)	8 (29.62)		1 (3.70)		9 (33.33)
41 - 50	2 (2.27)	0 (0)		0 (0)		0 (0)
Residence			0.968		0.654	
Urban	52 (59.10)	20 (38.46)		1 (1.92)		21 (40.38)
Rural	36 (40.90)	14 (38.88)		1 (2.77)		15 (41.66)
Education level			0.887		0.595	
Diploma or lower	68 (77.27)	26 (38.23)		2 (2.94)		28 (41.17)
University degree	20 (22.73)	8 (40.00)		0 (0)		8 (40.00)
Contact with cat			0.098		0.598	
Yes	32 (36.37)	16 (50.00)		1 (3.12)		17 (53.12)
No	56 (63.63)	18 (32.14)		1 (1.78)		19 (33.62)
Consumption of raw/undercooked meat			0.888		0.68	
Yes	38 (43.18)	15 (39.47)		1 (2.63)		16 (42.10)
No	50 (56.82)	19 (38.00)		1 (2.00)		20 (40.00)
Source of drinking water			0.015		0.538	
Purified water	60 (68.18)	18 (30.00)		1 (1.66)		19 (31.66)
Unpurified water	28 (31.82)	16 (57.14)		1 (3.57)		17 (60.71)
Reason for referral			0.37		0.175	
Routine checkup	80 (90.90)	30 (37.50)		1 (1.25)		31 (38.75)
Abortion	8 (9.10)	4 (50.00)		1 (12.50)		5 (62.50)
History of abortion			0.267		0.369	
Yes	18 (20.45)	9 (50.00)		1 (5.55)		10 (55.55)
No	70 (79.55)	25 (35.71)		1 (1.42)		26 (37.14)

^a Values are expressed as No. (%).^b P-value < 0.05 indicates a significant difference in seroprevalence between the categories within each characteristic.

Mazandaran province in northern Iran. The authors highlighted the importance of increasing knowledge about the main transmission routes of *T. gondii* infection among pregnant women and performing regular screening tests by an appropriate method (26). The seroprevalence rate of pregnant women in the current study was similar to the one reported by Foroutan-Rad et al. (26), and slightly higher than the rate reported by Rostami et al. (10). It is worth mentioning that the IgG seroprevalence of pregnant women drinking unpurified water was higher than those drinking purified water (57.14% vs. 30.00%; $P = 0.015$). This indicates that contaminated water is considered as a po-

tential source of transmission of *T. gondii* infection in this region. In our previous study (Abadan county, Khuzestan province), the same results were observed among the general population (24).

5.1. Conclusions

Based on our findings, the overall seroprevalence of 40.90% (36/88) to *T. gondii* infection was estimated during pregnancy period in Khorramshahr county. Since the risk of acquiring acute *T. gondii* infection in this susceptible group is clinically significant, improved prevention and control efforts should be strictly performed. Also,

the regular screening programs for *T. gondii* infection into the routine clinical care for pregnant women can be helpful. Finally, increased knowledge of women regarding *T. gondii* infection and its consequences, the main transmission routes, including source of drinking water, and the related risk factors could reduce the seroprevalence rate, especially in rural communities.

Acknowledgments

The authors sincerely appreciate all personnel of Valiasr hospital in Khorramshahr for their kind cooperation. We are very grateful to Mrs. Fatemeh Maghsoudi (Abadan University of Medical Sciences, Abadan, Iran) for her helpful consultation and comments on the manuscript.

Footnotes

Authors' Contribution: SS and MF designed the study protocol; SS, MS, MSK, MP, and MF collected the data and were involved in statistical analysis; SS performed the experiments; MF drafted the manuscript. SS and ADG critically revised the manuscript. All authors read and approved the final version of the manuscript.

Conflict of Interests: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data Reproducibility: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval: The study was approved by the Ethical Committee of Behbahan Faculty of Medical Sciences (IR.BHN.REC.1399.007). Ethical issues (including plagiarism, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) were completely observed by the authors.

Funding/Support: This study was financially supported by Grant No: 98113 from the Behbahan Faculty of Medical Sciences, Behbahan, Iran. The funders of this study had no role in the study design, analysis and interpretation of data, writing of the final paper, and the decision to submit the manuscript for publication. The corresponding authors had access to the data in the study and had final responsibility for the decision to submit for publication.

Informed Consent: All subjects voluntarily agreed to participate. Written informed consent was obtained from all subjects.

References

- Dubey JP. The history of *Toxoplasma gondii*—the first 100 years. *J Eukaryot Microbiol*. 2008;**55**(6):467–75. doi: [10.1111/j.1550-7408.2008.00345.x](https://doi.org/10.1111/j.1550-7408.2008.00345.x). [PubMed: [19120791](https://pubmed.ncbi.nlm.nih.gov/19120791/)].
- Fallahi S, Rostami A, Nouroollahpour Shiadeh M, Behniafar H, Paktinat S. An updated literature review on maternal-fetal and reproductive disorders of *Toxoplasma gondii* infection. *J Gynecol Obstet Hum Reprod*. 2018;**47**(3):133–40. doi: [10.1016/j.jogoh.2017.12.003](https://doi.org/10.1016/j.jogoh.2017.12.003). [PubMed: [29229361](https://pubmed.ncbi.nlm.nih.gov/29229361/)].
- Montoya JG, Remington JS. Management of *Toxoplasma gondii* infection during pregnancy. *Clin Infect Dis*. 2008;**47**(4):554–66. doi: [10.1086/590149](https://doi.org/10.1086/590149). [PubMed: [18624630](https://pubmed.ncbi.nlm.nih.gov/18624630/)].
- Khademvatan S, Foroutan M, Hazrati-Tappeh K, Dalvand S, Khalkhali H, Masoumifard S, et al. Toxoplasmosis in rodents: A systematic review and meta-analysis in Iran. *J Infect Public Health*. 2017;**10**(5):487–93. doi: [10.1016/j.jiph.2017.01.021](https://doi.org/10.1016/j.jiph.2017.01.021). [PubMed: [28237696](https://pubmed.ncbi.nlm.nih.gov/28237696/)].
- Rostami A, Riahi SM, Contopoulos-Ioannidis DG, Gamble HR, Fakhri Y, Shiadeh MN, et al. Acute *Toxoplasma* infection in pregnant women worldwide: A systematic review and meta-analysis. *PLoS Negl Trop Dis*. 2019;**13**(10). e0007807. doi: [10.1371/journal.pntd.0007807](https://doi.org/10.1371/journal.pntd.0007807). [PubMed: [31609966](https://pubmed.ncbi.nlm.nih.gov/31609966/)], [PubMed Central: [PMC6822777](https://pubmed.ncbi.nlm.nih.gov/PMC6822777/)].
- Wang ZD, Liu HH, Ma ZX, Ma HY, Li ZY, Yang ZB, et al. *Toxoplasma gondii* Infection in Immunocompromised Patients: A Systematic Review and Meta-Analysis. *Front Microbiol*. 2017;**8**:389. doi: [10.3389/fmicb.2017.00389](https://doi.org/10.3389/fmicb.2017.00389). [PubMed: [28337191](https://pubmed.ncbi.nlm.nih.gov/28337191/)]. [PubMed Central: [PMC5343064](https://pubmed.ncbi.nlm.nih.gov/PMC5343064/)].
- Saki J, Foroutan M, Khodkar I, Khodadadi A, Nazari L. Seroprevalence and molecular detection of *Toxoplasma gondii* in healthy blood donors in southwest Iran. *Transfus Apher Sci*. 2019;**58**(1):79–82. doi: [10.1016/j.transci.2018.12.003](https://doi.org/10.1016/j.transci.2018.12.003). [PubMed: [30579749](https://pubmed.ncbi.nlm.nih.gov/30579749/)].
- Maleki B, Ahmadi N, Olfatifar M, Gorgipour M, Taghipour A, Abdoli A, et al. *Toxoplasma* oocysts in the soil of public places worldwide: a systematic review and meta-analysis. *Trans R Soc Trop Med Hyg*. 2021;**115**(5):471–81. doi: [10.1093/trstmh/traa133](https://doi.org/10.1093/trstmh/traa133). [PubMed: [33205208](https://pubmed.ncbi.nlm.nih.gov/33205208/)].
- Foroutan M, Rostami A, Majidiani H, Riahi SM, Khazaei S, Badri M, et al. A systematic review and meta-analysis of the prevalence of toxoplasmosis in hemodialysis patients in Iran. *Epidemiol Health*. 2018;**40**. e2018016. doi: [10.4178/epih.e2018016](https://doi.org/10.4178/epih.e2018016). [PubMed: [29748456](https://pubmed.ncbi.nlm.nih.gov/29748456/)]. [PubMed Central: [PMC6060338](https://pubmed.ncbi.nlm.nih.gov/PMC6060338/)].
- Rostami A, Riahi SM, Gamble HR, Fakhri Y, Nouroollahpour Shiadeh M, Danesh M, et al. Global prevalence of latent toxoplasmosis in pregnant women: a systematic review and meta-analysis. *Clin Microbiol Infect*. 2020;**26**(6):673–83. doi: [10.1016/j.cmi.2020.01.008](https://doi.org/10.1016/j.cmi.2020.01.008). [PubMed: [31972316](https://pubmed.ncbi.nlm.nih.gov/31972316/)].
- Foroutan-Rad M, Majidiani H, Dalvand S, Daryani A, Kooti W, Saki J, et al. Toxoplasmosis in Blood Donors: A Systematic Review and Meta-Analysis. *Transfus Med Rev*. 2016;**30**(3):116–22. doi: [10.1016/j.tmr.2016.03.002](https://doi.org/10.1016/j.tmr.2016.03.002). [PubMed: [27145927](https://pubmed.ncbi.nlm.nih.gov/27145927/)].
- Pappas G, Roussos N, Falagas ME. Toxoplasmosis snapshots: global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. *Int J Parasitol*. 2009;**39**(12):1385–94. doi: [10.1016/j.ijpara.2009.04.003](https://doi.org/10.1016/j.ijpara.2009.04.003). [PubMed: [19433092](https://pubmed.ncbi.nlm.nih.gov/19433092/)].
- Torgerson PR, Mastroiacovo P. The global burden of congenital toxoplasmosis: a systematic review. *Bull World Health Organ*. 2013;**91**(7):501–8. doi: [10.2471/BLT.12.111732](https://doi.org/10.2471/BLT.12.111732). [PubMed: [23825877](https://pubmed.ncbi.nlm.nih.gov/23825877/)]. [PubMed Central: [PMC3699792](https://pubmed.ncbi.nlm.nih.gov/PMC3699792/)].
- Sharbatkhori M, Dadi Moghaddam Y, Pagheh AS, Mohammadi R, Hedayat Mofidi H, Shojaee S. Seroprevalence of *Toxoplasma gondii* Infections in Pregnant Women in Gorgan City, Golestan Province, Northern Iran-2012. *Iran J Parasitol*. 2014;**9**(2):181–7. [PubMed: [25848383](https://pubmed.ncbi.nlm.nih.gov/25848383/)]. [PubMed Central: [PMC4386037](https://pubmed.ncbi.nlm.nih.gov/PMC4386037/)].
- Hoseini SA, Dehgani N, Sharif M, Daryani A, Gholami S, Ebrahimi F. Serological survey of toxoplasmosis in pregnant women. *J Mazandaran Univ Med Sci*. 2014;**24**:146–50.

16. Rasti S, Hooshyar H, Arbabi M, Fatahian A, Behrashi M, Talebian A, et al. Frequency of Toxoplasma Infection Among Pregnant Women and Their Newborn in Kashan, Iran. *Zahedan J Res Med Sci.* 2015;**17**(6). doi: [10.17795/zjrms999](https://doi.org/10.17795/zjrms999).
17. Akhlaghi L, Shirbazou S, Maleki F, Keyghobadi A, Tabaraei Y, Tabatabaie F. Seroepidemiology of toxoplasma infection in pregnant women in Qom Province, Iran (2010). *Life Sci.* 2013;**10**(SUPPL):322-5.
18. Fallah M, Rabiee S, Matini M, Taherkhani H. Seroepidemiology of toxoplasmosis in primigravida women in Hamadan, Islamic Republic of Iran, 2004. *East Mediterr Health J.* 2008;**14**(1):163-71.
19. Hajsoleimani F, Ataiean A, Nourian AA, Mazloomzadeh S. Seroprevalence of Toxoplasma gondii in pregnant women and bioassay of IgM positive cases in Zanjan, Northwest of Iran. *Iran J Parasitol.* 2012;**7**(2):82-6.
20. Soltani S, Ghaffari AD, Kahvaz MS, Sabaghan M, Pashmforosh M, Foroutan M. Detection of Anti-Toxoplasma gondii IgG and IgM Antibodies and Associated Risk Factors during Pregnancy in Southwest Iran. *Infect Dis Obstet Gynecol.* 2021;**2021**:5547667. doi: [10.1155/2021/5547667](https://doi.org/10.1155/2021/5547667). [PubMed: [34135564](https://pubmed.ncbi.nlm.nih.gov/34135564/)]. [PubMed Central: [PMC8175175](https://pubmed.ncbi.nlm.nih.gov/PMC8175175/)].
21. Saki J, Mohammadpour N, Moramezi F, Khademvatan S. Seroprevalence of Toxoplasma gondii in women who have aborted in comparison with the women with normal delivery in Ahvaz, southwest of Iran. *Sci World J.* 2015;**2015**:764369. doi: [10.1155/2015/764369](https://doi.org/10.1155/2015/764369). [PubMed: [25699288](https://pubmed.ncbi.nlm.nih.gov/25699288/)]. [PubMed Central: [PMC4325198](https://pubmed.ncbi.nlm.nih.gov/PMC4325198/)].
22. Firooz Jahantigh F, Rasekh Ganjali M, Sarani A. [Seroprevalence of Toxoplasma gondii infection among pregnant women and small ruminant populations in Sistan region, Iran.]. *Iran J Vet Med.* 2020;**14**:239-49. Persian.
23. Ebrahimzadeh A, Mohammadi S, Salimi-Khorashad A, Jamshidi A. Seroprevalence of toxoplasmosis among pregnant women referring to the reference laboratory of Zahedan, Iran. *Zahedan J Res Med Sci.* 2013;**15**:32-5.
24. Soltani S, Foroutan M, Afshari H, Hezarian M, Kahvaz MS. Seroepidemiological evaluation of Toxoplasma gondii immunity among the general population in southwest of Iran. *J Parasit Dis.* 2018;**42**(4):636-42. doi: [10.1007/s12639-018-1047-2](https://doi.org/10.1007/s12639-018-1047-2). [PubMed: [30538365](https://pubmed.ncbi.nlm.nih.gov/30538365/)]. [PubMed Central: [PMC6261153](https://pubmed.ncbi.nlm.nih.gov/PMC6261153/)].
25. Soltani S, Kahvaz MS, Soltani S, Maghsoudi F, Foroutan M. Seroprevalence and associated risk factors of Toxoplasma gondii infection in patients undergoing hemodialysis and healthy group. *BMC Res Notes.* 2020;**13**(1):551. doi: [10.1186/s13104-020-05396-5](https://doi.org/10.1186/s13104-020-05396-5). [PubMed: [33287882](https://pubmed.ncbi.nlm.nih.gov/33287882/)]. [PubMed Central: [PMC7720589](https://pubmed.ncbi.nlm.nih.gov/PMC7720589/)].
26. Foroutan-Rad M, Khademvatan S, Majidiani H, Aryamand S, Rahim F, Malehi AS. Seroprevalence of Toxoplasma gondii in the Iranian pregnant women: A systematic review and meta-analysis. *Acta Trop.* 2016;**158**:160-9. doi: [10.1016/j.actatropica.2016.03.003](https://doi.org/10.1016/j.actatropica.2016.03.003). [PubMed: [26952970](https://pubmed.ncbi.nlm.nih.gov/26952970/)].