Review Article

More Than Seventy Years of Research (1948–November 2021) on Toxoplasma gondii in Iran: A Narrative Review

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Abstract

In this review, we intend to provide a summary of the activities of researchers in the field of Toxoplasma gondii in Iran, during the past 70 years. Most studies have been limited to epidemiological studies (mostly using ELISA and IFA methods). Designing a standard and reliable method using the specific antigens of this parasite is essential. So far, studies in the field of drug effects have not been able to introduce an effective drug with few side effects. Various types of vaccines have been developed, such as recombinant and DNA vaccines. However, none of them had a good efficacy. The use of multi-epitope vaccines as potential vaccines against toxoplasmosis is recommended. At present, limited studies have been conducted on the patterns of transmission and genetic diversity of isolated isolates in Iran. Future research to determine the genotype of T. gondii could play an important role in the study of population structure, and biological characteristics of this parasite. It is hoped that the results of this study will help control, prevent, and reduce the burden of disease caused by this parasite.

Keywords:
Review; Toxoplasma gondii; Iran

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Introduction

Toxoplasma gondii is an obligate intracellular parasite and infection a wide range of hosts, containing humans and animals. T. gondii infects about one third of the world's population (1-4). Infection with T. gondii is acquired by eating food contaminated
with sporulated oocysts and consuming of raw or undercooked meat infected to tissue cysts. Vertical transmission also occurs, and iatrogenic transmission may happen via organ transplant (5-7).

Considering the importance of this parasite in the congenital toxoplasmosis, the development of opportunistic infection in immunodeficiency individuals, as well as climatic and social conditions in Iran, it is necessary to provide comprehensive information about the situation of toxoplasmosis. In Iran, various studies have been conducted in the fields of epidemiology, pathogenesis, treatment, vaccines preparation, etc. However, a review study is necessary to collect and provide a summary of relevant data to provide proper solutions for the prevention, control, diagnosis and treatment of toxoplasmosis in Iran. It also makes future studies more targeted.

We aimed to give a summary of toxoplasmosis status in Iran from 1948 through 2021 in various fields of epidemiology, determination of genotypes, diagnosis, drug treatment, and vaccine, as well as the role of the environment and food factors in the transmission of infection.

**Search strategy and primary finding**

To collect data, a comprehensive search was performed on all scientific publications in 4 English (PubMed, ScienceDirect, Google Scholar and Scopus) and 4 Persian databases (Magiran, IranDoc, IranMedex and SID) from 1948 to Nov 2021. The search applied the following keywords: "Toxoplasma gondii", "toxoplasmosis", and "Iran". The evaluation of studies was performed according to the inclusion and exclusion criteria. Inclusion criteria: (a) All English and Persian studies related to T. gondii with Iranian Affiliation. Exclusion criteria: (a) All studies related to T. gondii with non-Iranian affiliation; (b) Articles published in non-English and non-Persian languages; (c) Papers presented at scientific conferences and congresses.

**Overview of conducted articles based on subject**

The articles published on T. gondii from 1948 to 2021 numbered about 1540, included 296 (19.22%) in Persian and 1244 (80.78%) in English languages. Frequency of published papers based on subject in Iran is shown in Fig. 1.
History
The population of Iran in 2021 was estimated over 83 million (https://www.amar.org.ir/). Iran has four geographical regions comprising 31 provinces. Also, Iran has a long history of research on T. gondii, dates back to 1948, and Ansari reported the first case of the disease. The first research on toxoplasmosis in Iran was performed using Sabin-Feldman test in 1954 (1). Jamalian et al. conducted the first clinical trial of T. gondii in 1973 (published 1974) (2). Presence of anti-T. gondii antibodies in Iranian society has been reported in several studies. The results indicate difference in prevalence of infection in different geographical areas; the lowest rate (12%) was in Khuzestan and the highest rate in Mazandaran (87.5%) (3).

Epidemiological studies
Epidemiological studies (868) covered 616 (70.97%) human subjects and 252 (29.03%) animal subjects.

Human Epidemiological studies
Epidemiological studies of T. gondii on human (616/1540;4%) are divided into two groups: healthy group (56.81%) including pregnant women, girls before marriage, blood donors, etc., and the clinical cases (43.19%) including AIDS, cancer, Parkinson, schizophrenia, etc. (3-7) (Table 1).

| Group            | Epidemiological studies | NO. articles | % of study | % T. gondii |
|------------------|-------------------------|--------------|------------|-------------|
| Human            | General population      | 186/616      | 30.2       | 39.3        |
|                  | Pregnant women          | 133/616      | 21.6       | 41          |
|                  | Neonates                |              | 0.64       |             |
|                  | Infants                 | 18/616       | 2.92       | 4.1         |
|                  | Suspected of CT         |              |            |             |
|                  | Blood donor             | 24/616       | 3.9        | 34.4        |
|                  | Cancer                  | 30/616       | 4.88       | 45.06       |
|                  | HIV+                    | 34/616       | 5.51       | 50.05       |
|                  | Parkinson               | 3/616        | 0.49       | 53 to 85    |
|                  | Hemodialysis            | 29/616       | 4.70       | 58          |
|                  | Diabetic                | 15/616       | 2.43       | 35.1 to 60.43 |
|                  | Schizophrenia           | 14/616       | 2.27       | 34 to 72.5  |
|                  | Alzheimer               | 5/616        | 0.81       | 61.3 to 66.6 |
|                  | Ocular                  | 10/616       | 1.62       | 8.36 to 82.5   |
|                  | Mental disorders        | 32/616       | 5.2        | -           |
| Animals          | Sheep and goats         | 97/252       | 38.5       | 31 and 27   |
| Cattle           |                         | 38/252       | 15.07      | 18.1        |
| Cats             |                         | 33/252       | 13.1       | 33.6        |
| Birds            |                         | 22/252       | 8.73       | 16.52       |
| Rodents          |                         | 16/252       | 6.35       | 15          |
| Camels           |                         | 7/252        | 2.78       | 9.93 to 28.06 |
| Dogs             |                         | 10/252       | 3.97       | 10.1 to 77  |
| Horses           |                         | 8/252        | 3.17       | 13.3 to 71.2 |
| Cold-blooded     |                         | 3/252        | 1.19       | Fish (10), Snakes (80.88), seals (83) |
Animal Epidemiological studies

Overall, 252 studies have been performed on the prevalence of toxoplasmosis in animals, as shown in Table 1 (8-11).

Animal food products

Twenty studies (1.3%) have been conducted on food including meat, milk and animal products as well as eggs. In the meantime, 10 studies were performed on meat which showed prevalence rate of 4-24% (12, 13). Moreover, 9 studies were performed on milk samples that prevalence was reported 5.4%-11.38% (14, 15) and there was also a study on domestic and industrial eggs. Based on the results of this study, T. gondii DNA was detected in 11% of the eggs (16).

Environment

There have been few studies on the contamination of the soil and water with T. gondii oocysts in Iran. Four studies have been performed to diagnose presence of T. gondii DNA in soil and water in Tehran, Ahvaz, Arak, Guilan and Mazandaran provinces (prevalence 5.8% to 78.1%) (17-21).

Development of diagnostic approach of T. gondii

Iranian scientists have conducted several studies (10.39%) to develop diagnostic methods for T. gondii infection. Diagnostic tests are mainly three groups, including serological tests detecting T. gondii antibodies, and antigens, and molecular methods (DNA detection) (22).

In the last two decades, Iranian researchers have studied the effects of various T. gondii antigens, including recombinant antigens on the improvement of serological test, and difference between acute and chronic infection. SAG1, SAG2, SAG3, GRA2, GRA6, GRA7, GRA8, ROP1, SRS3, and ESA are among the antigens examined whose details are presented in Table 2. Overall, the results showed that the use of antigens such as SAG1, GRA7, and GRA6 for serodiagnosis of acute and chronic toxoplasmosis in human sera could be very effective. Javadi et al. (2020) conducted a bioinformatics study on multi-epitope antigens SAG1, GRA7 and ROP1 showed that the use of the antigens can be considered as a diagnostic kit for acute and chronic Toxoplasmosis (23).

The T. gondii genetic diversity and its population structures in Iran

The genetic characteristics of the reported studies in Iran are summarized in Table 3. In total DNA or isolates separated in 270 animals, 145 humans, 66 meats, and 31 soil samples were analyzed in the 20 studies in Iran. The isolates were often typed by PCR-RFLP based on SAG2 and GRA6 genes. The typing results based on PCR-RFLP and microsatellite markers among the 505 DNA or isolates separated from different hosts revealed Type III (32.62%) had the highest frequency, mix and atypical types had the lowest frequency in Iran. Out of the remaining samples, Type I and II had the frequency rates of 31.64% and 26.76%, respectively. There are no reports on the genetic diversity with more than five typing markers, and only one study has been done by Hosseini et al. (2019) on HIV+ patients with multilocus PCR-RFLP method using 12 genes (44). It is believed that using low or limited markers would not provide reliable results on population structures (45). However, identification of the genetic groups could provide a key role to play in studies on the population structure, epidemiology and biological characteristics of T. gondii.
Table 2: Summary of antigen/peptides used in design of ELISA kit by researchers in Iran

| References | Method      | Gen/Peptide | Sensitivity (%) | Specificity (%) |
|------------|-------------|-------------|-----------------|-----------------|
| (24)       | ELISA       | GRA2        | 100 (IgG)       | 96.4 (IgG)      |
|            |             |             | 71.4 (IgM)      |                 |
| (25)       | ELISA       | GRA6        | 87.5 (IgG)      | 94.1 (IgG)      |
| (26)       | ELISA       | SAG1, 30KD  | 94.5 (IgG)      | 93.6 (IgG)      |
| (27)       | ELISA       | ESA         | 84 (IgG)        | 92 (IgG)        |
| (28)       | ELISA       | SAG1        | 88.4 (IgG)      | 88 (IgG)        |
| (29)       | ELISA avidity | GRA6   | 85 (IgG)        | 100 (IgG)       |
|            |             |             | 100 (IgM)       |                 |
| (30)       | ELISA       | SAG1        | 100 (IgG)       | 96 (IgG)        |
| (31)       | ELISA       | GRA7        | 89 (IgG)        | 90 (IgG)        |
|            |             |             | 96 (IgM)        | 90 (IgM)        |
| (32)       | ELISA       | SAG1        | 93 (IgG)        | 95 (IgG)        |
|            |             |             | 87 (IgM)        |                 |
| (33)       | ELISA       | SAG1        | 80 (IgM)        | 90 (IgM)        |
| (34)       | ELISA       | SAG1        | 93.6 (IgG)      | 92.9 (IgG)      |
|            |             |             | 39.3 (IgM)      | 80 (IgM)        |
|            |             |             | SAG2 100 (IgG)  | 89.4 (IgG)      |
|            |             |             | 64.3 (IgM)      | 83.3 (IgM)      |
|            |             |             | SAG3 95.4 (IgG) | 91.2 (IgG)      |
|            |             |             | 17.9 (IgM)      | 76.7 (IgM)      |
| (35)       | ELISA       | SAG1        | 94.1 (IgG)      | 100 (IgG)       |
|            |             |             | 100 (IgM)       |                 |
| (36)       | ELISA       | r ROP1      | 92 (IgG)        | 94 (IgG)        |
| (37)       | ELISA       | r GRA7      | 84.6 (IgG)      | -               |
|            |             |             | 86.9 (IgM)      |                 |
| (38)       | ELISA avidity | GRA8     | 92 (IgG)        | 96 (IgG)        |
|            |             |             | 82.89 (IgG)     | 91 (IgG)        |
| (39)       | ELISA       | r SAG1      | 100 (IgG)       |                 |
| (40)       | ELISA       | RT-SRS3     | 83.7 (IgG)      | 90.2 (IgG)      |
|            |             |             | 81.2 (IgM)      | 89.3 (IgM)      |
| (41)       | Flow immune assay | r GRA7 | 100 (IgG)       | 96.7 (IgG)      |
| (42)       | Dot-Elisa   | r SAG1      | 66.2 (IgG)      | 81.2 (IgG)      |
|            |             |             | 87.5 (IgM)      | 83.9 (IgM)      |
|            |             |             | r SAG1 + r GRA7 | 86.2 (IgG)      |
|            |             |             | 90.6 (IgM)      | 92 (IgM)        |
Table 3: The genetic characterization of *Toxoplasma gondii* isolates from different hosts in Iran

| Ref. | Host | No. of DNA/Isolates | Molecular marker(s) | No. Type I (%) | No. Type II (%) | No. Type III (%) | No. mix and atypical (%) |
|------|------|---------------------|---------------------|----------------|----------------|---------------------|--------------------------|
| (46) | Human | 13                  | SAG2                | 1 (7.6)        | 12 (92.4)      | -                   | -                        |
|      | Rodent | 8                   |                     | -              | 2 (25)         | 6 (75)             | -                        |
| (47) | Sheep | 4                   | TUB2, W35, TgM-A, B18, and B17 | -              | 2              | 2                   | -                        |
|      | Bird   | 7                   |                     | -              | -              | 7                   | -                        |
|      | Cat    | 2                   |                     | -              | 2              | -                   | -                        |
|      | Human  | 3                   |                     | -              | 2              | 1                   | -                        |
| (48) | Aborted fetuses (Sheep) | 12 | B1 | 12 (100) | - | - | - |
| (12) | Meat product | 40 | SAG2 | - | 40 (100) | - | - |
| (49) | Aborted fetuses (Ovine) | 0 | GRA6 | - | - | - | - |
| (17) | Soil | 13 | SAG2 | 1 (7.7) | - | 8 (61.5) | 4; mix I&III (30.8) |
| (50) | Bird | 41 | GRA6 | - | 8 (19.5) | 33 (80.5) | - |
| (51) | Aborted fetuses (Human) | 65 | SAG2 | 11 (16.9) | 54 (83.1) | - | - |
| (52) | Human | 52 | GRA6 | - | - | 52 (100) | - |
| (18) | Soli | 18 | GRA6 | - | 6 (33.3) | 12 (66.7) | - |
| (53) | Wild boar | 5 | Sequencing with 529 | 1 (20) | - | 4 (80) | - |
| (54) | Aborted fetuses (Ovine) | 5 | GRA6 | 5 (100) | - | - | - |
| (55) | Cat | 35 | SAG2 | 1 (2.9) | - | 32 (91.4) | 2; mix I&III (5.7) |
| (56) | Aborted fetuses (Ewe) | 10 | SAG2, SAG3, and GRA6 | 3 (30) | 2 (20) | - | 5 atypical (50) |
| (13) | Chicken meat | 4 | SAG2 | 4 (100) | - | - | - |
|      | Beef meat | 8 | - | 8 (100) | - | - | - |
|      | Lamb meat | 14 | - | 14 (100) | - | - | - |
| (57) | Aborted fetuses (Human) | 2 | SAG3, GRA6 | - | - | 2 (100) | - |
Treatment studies for *T. gondii*

Very few investigations (n= 100, 6.49 %) have been carried out on the treatment of *T. gondii* infection in Iran. The efficacy of synthetic drugs (62%), herbal medicines and other compounds (38%) against *T. gondii* were evaluated in vitro and in vivo. The synthetic drugs, such as propranolol, Atovaquone, Clindamycin, Co-trimoxazole, etc., have shown good anti-toxoplasmic effects (61). Recently, attention has been drawn to the therapeutic potential of herbal products in Iran due to their lower side effects and higher availability. According to a systematic review, the extracts of Garlic, *Achilleamillefolium*, *Hypericum perforatum*, *Sambucus nigra* were tested for the treatment of toxoplasmosis (62).

Most of the studies were performed on the acute toxoplasmosis using RH strain. Since currently available treatments of toxoplasmosis are insufficiently effective with severe side effects, new therapeutic options for the treatment are urgently needed that effective penetration and concentration in the placent, trans placental passage, parasitical properties versus the different parasitic stages, penetration into cysts, and distribution in the main sites.

Immunization studies against *T. gondii*

In total, 183 studies (11.88%) on immunization and vaccination against *T. gondii* have been conducted in Iran. Many researchers conducted the investigations regarding the vaccine types against toxoplasmosis. Various strategies have been used to evaluate vaccines against toxoplasmosis including live attenuated vaccines, DNA vaccines, recombinant protein vaccines and epitope-based vaccines. The main outcomes of vaccines evaluation have been summarized in Table 4.

| (58) | Sheep | 125 | SAG2, and GRA6 | 90 (72) | - | 3 (2.4) | 9 mix I&II (7.2), 21 I&III (16.8), 1 II&III (0.8), 1 I,II,III (0.8) |
| (59) | Hooded crow | 9 | GRA6 | - | - | 9 (100) | 1 #35 or I variant (10), 1 #27 or I variant (10), 1 #48 or III variant (10) |
| (44) | AIDS | 10 | SAG1,SAG2,SAG3,alt-SAG2,BTUB,GRA6,Apico,PK1,C22-8,C29-2,CS3 | 2 (20) | 3 (30) | 2 (20) | 1 #35 or I variant (10), 1 #27 or I variant (10), 1 #48 or III variant (10) |
| (60) | Sheep and Cattle | 7 | GRA6,B1 | 7(100) | | | |
| Total | - | 512 | TUB2, W35, TgM-A, B18, B17, B1, Sequencing with 529, SAG2, SAG3, SAG1, alt-SAG2, BTUB, GRA6, Apico, PK1, C22-8, C29-2, CS3 | 162 (31.64) | 137 (26.7) | 167 (32.62) | 46 (8.98) |
| Variable                      | Parasite strain | Antigens/ adjuvant                  | Effect                                                                 | References |
|-------------------------------|-----------------|-------------------------------------|----------------------------------------------------------------------|------------|
| DNA vaccines                  | Not determined  | SAG1, SAG3, SAG5/ CpG-ODN           | Increased survival time 10 and fewer parasite load (15,485 per mg of spleen). | (63)       |
|                               |                 | # ROP38                             | ROP38 was proved a non-allergenic and antigenic protein. It had Sec signal peptide (Sec/SPI) with 0.8762 likelihood. It is suitable candidate vaccine against toxoplasmosis. | (64)       |
| RH                            | ROP13 in pcDNA3 Plasmid | ROP13 was successfully sub cloned into the pcDNA3 expression vector. |                                                            | (65)       |
| $2 \times 10^3$ RH            | ROP8 +IL 12     | Enhanced the level of anti-\textit{T. gondii} antibodies. |                                                            | (66)       |
|                               | SAG1-Related Sequence 3 (SRS3) | SRS3 stimulate the immune system against toxoplasmosis. |                                                            | (67)       |
|                               | # ROP16         | This protein was immunogenic and non-allergenic. |                                                            | (68)       |
|                               | # GRA12         | GRA12 had several excellent B-cells and T-cells epitope, indicating that it would become an excellent vaccine against \textit{T. gondii}. |                                                            | (69)       |
|                               | # SAG1          | Analysis showed several immunodominant B-cell, cytotoxic and Helper T-lymphocyte epitopes with excellent immunogenicity properties, rendering it as a prominent vaccine candidate. |                                                            | (70)       |
| Recombinant protein vaccines  | RH              | rSAG1-loaded PLGA                   | Elicited higher IFN-\gamma, specific anti-\textit{T.gondii} IgG and longer survival time in mice. | (71)       |
Inactivated parasite, crude or purified antigens

| Multi-epitope vaccine | RH       | Th17/GRA14 and ROP13 | Enhanced of IL-17, and IL-22 and significant induction in ROS and considerable decrease in parasite load was observed in mice. |
|-----------------------|----------|----------------------|-----------------------------------------------------------------------------------------------------------------|
| 2 × 10³ RH            | ROP8     |                      | Induced strong humoral and cellular responses and prolonged the survival time in BALB/c. |
| 1 × 10⁴ RH            | MIC3, ROP8, and SAG1 |                      | Effective protection against the parasite achieved an increase in survival time in the immunized mice, especially in the MRS-CaNP group. |

Inactivated parasite, crude or purified antigens

| # Calcium-Dependent Protein Kinase 7 (CDPK7) | The protein has immunogenic and nonallergenic nature. |
| # calcium-dependent protein kinase-3 (CDPK3) | It is higher affinity for MHC-binding and CTL epitopes |
| 1.5 × 10⁶ RH Soluble total antigen | STAg increased the release of IL1β. IL18 significantly upregulated after 24 h. |

#: Bioinformatics investigation

In general, killed *T. gondii* parasite vaccines cannot be effective enough in any of the infection animal models. In contrast, live-attenuated vaccines are capable of enhancing MHC class I-restricted CD8⁺ T-cell immune responses, which it is considered as the most important pathway for the elimination of intracellular parasites. However, there are major concerns that attenuated vaccines have been the risk of reverting to a pathogenic strain. Currently, appropriate antigens from different parts of *T. gondii* are used as DNA vaccine or recombinant protein vaccine, which stimulates immune responses against *T. gondii* infection. Antigens used as suitable candidates for immunization studies include SAG1, SAG2, SAG3, GRA2, GRA5, GRA6, GRA7, GRA8, GRA14, ROM4, ROP1, ROP2, BAG1 and MIC3. Protein prime/DNA boost have been shown as an efficient strategy to induce both cellular and humoral immune. Recently the use of multi-epitope vaccines has become popular in research institutes in Iran as new potential vaccines against toxoplasmosis. Multi-antigenic vaccinations could overcome for limitation of single antigen and enhance the protective immunity against *T. gondii* infection.

**Discussion**

More than 70 years have passed since the first case of clinical detection of toxoplasmosis in Iran. The relatively high prevalence of *T. gondii*...
gondii infection in animals, especially farm animals as the main sources of meat consumed by the Iranian people, as well as environmental pollution (water and soil), increases the risk of human infections. The prevalence of toxoplasmosis in the general population of Iran is reported to be 39% which may be due to geographical location and habitat, eating habits, and lifestyle. Most diagnostic studies in Iran are based on the diagnosis of IgM and IgG anti-Toxoplasma antibodies which today due to false positive and false negative test results especially in pregnant women, their results are controversial. Efforts to establish a fully standardized diagnostic method with high sensitivity and specificity are needed to dissolve this problem using specific antigens of T. gondii and designing diagnostic kits. Information on genetic diversity of the parasite in human and animal toxoplasmosis in Iran using the Mn-PCR-RFLP method is highly limited; however, ToxoDB genotyping of T. gondii can play an important role in studies on population structure, epidemiology, vaccine, and biological characteristics of T. gondii. Over the years, there have been a few studies on the effects of drugs and synthetic compounds, as well as plant extracts on T. gondii. So far, there has been no effective drug to treat and prevent toxoplasmosis with low side effects; although none of the drugs have been effective on the cyst form of T. gondii. Numerous studies have been conducted in the field of designing different models of vaccine candidates. These include live-attenuated vaccines, DNA vaccines, recombinant protein vaccines, and multi-epitope vaccines that are useful for limiting single antigens and enhancing the protective immunity against T. gondii infection. Up to now, several studies using different diagnostic tests have shown the prevalence of T. gondii in cats in Iran. However, it is necessary to conduct a comprehensive study at the national level and with a single test on cats. To date, no national studies have been conducted on the GIS of Toxo-

plasma in humans, animals and environmental samples. Therefore, there is a need for a comprehensive study of toxoplasmosis in different populations across the country. This is important for providing information on areas where control efforts should be targeted. Toxoplasmosis is clearly neglected as a human disease, according to reports of the infection. Moreover, a comprehensive investigation has not been carried out in the field of awareness and health education (KAP study) among girls in schools and women on the verge of marriage. Hence, these studies are highly recommended to help prevent and control the infection. Moreover, the incidence of congenital toxoplasmosis in Iranian infants and pregnant women is unknown. Therefore, it is necessary to conduct a comprehensive study in the country to determine the parasitic burden of infection in pregnant women and infants. Future research in various fields of T. gondii, especially in the case of treatment (with emphasis on cystic form) and vaccination, is highly recommended for evaluating various drug derivatives and multi-epitope vaccine candidates.

Limitation

In this study, we reviewed all Persian and English articles related to T. gondii with Iranian affiliation, and therefore, according to the mentioned entry and exit criteria, no study has been lost. Due to the high volume of articles (1540) and also having a limit on the number of words and references for this journal, we tried to include the most important and up-to-date articles in the study (in table or references).

Conclusion

More than half of the conducted studies in Iran were in the field of epidemiology. In order to obtain sufficient information for proper control, prevention and treatment of toxoplasmosis in Iran, it is necessary to perform further studies in the field of genotyping, diagnosis, vaccination and treatment.
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Conflict of interest

None.

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