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Iranian Society of Parasitology
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Review Article

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

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Received 15 Jan 2021
Accepted 18 Mar 2022

Keywords:
Review;
Toxoplasma gondii;
Iran

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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.

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.

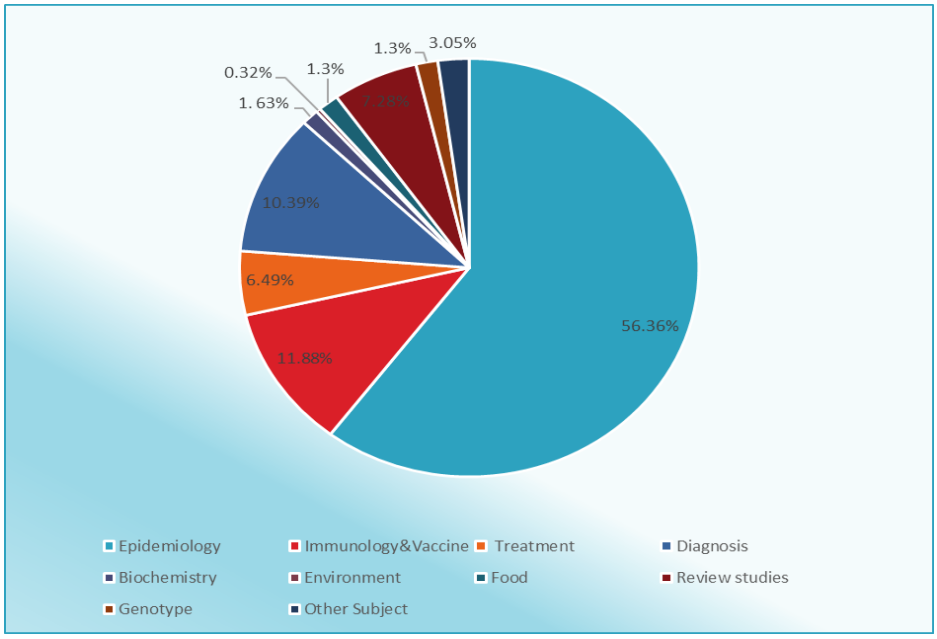

Fig. 1: Frequency of published papers based on subject in Iran since 1948-November 2021

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).

Table 1: Frequency of human and animal epidemiological studies in Iran

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	
	Suspected of CT			4.1
	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 of4-24% (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) 71.4 (IgM)	96.4 (IgG)
(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 (IgM)	100 (IgG)
(30)	ELISA	SAG1	100 (IgG)	96 (IgG)
(31)	ELISA	GRA7	89 (IgG) 96 (IgM)	90 (IgG) 90 (IgM)
(32)	ELISA	SAG1	93 (IgG) 87 (IgM)	95 (IgG)
(33)	ELISA	SAG1	80 (IgM)	90 (IgM)
(34)	ELISA	SAG1	93.6 (IgG) 39.3 (IgM)	92.9 (IgG) 80 (IgM)
		SAG2	100 (IgG) 64.3 (IgM)	89.4 (IgG) 83.3 (IgM)
		SAG3	95.4 (IgG) 17.9 (IgM)	91.2 (IgG) 76.7 (IgM)
(35)	ELISA	SAG1	94.1 (IgG) 100 (IgM)	100 (IgG)
(36)	ELISA	r ROP1		
(37)	ELISA	r GRA7	92 (IgG)	94 (IgG)
(38)	ELIS Avidity	GRA8	84.6 (IgG) 86.9 (IgM)	-
(39)	ELISA	r SAG1	92 (IgG)	96 (IgG)
(40)	ELISA	RT-SRS3	82.89 (IgG) 100 (IgM)	91 (IgG)
(41)	Flow immune assay	r GRA7	100 (IgG)	96.7 (IgG)
(42)	Dot-Elisa	r SAG1	83.7 (IgG) 81.2 (IgM)	90.2 (IgG) 89.3 (IgM)
		r GRA7	66.2 (IgG) 87.5 (IgM)	81.2 (IgG) 83.9 (IgM)
		r SAG1+rGRA7	86.2 (IgG) 90.6 (IgM)	91.1 (IgG) 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)	-

(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)	-
(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 (26.76)	167 (32.62)	46 (8.98)

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, Hypericumperforatum, Sambucusnigra* 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 placenta, 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.

Table 4 : Summary of *Toxoplasma gondii* vaccine candidates from 2020 designed by researchers in Iran

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)
	2×10^3 RH	ROP8 +IL 12	Enhanced the level of anti-*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 *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-γ, specific anti-T.gondii IgG and longer survival time in mice.	(71)

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.	(72)
	2×10^3 RH	ROP8	Induced strong humoral and cellular responses and prolonged the survival time in BALB/c.	(73)
	1×10^4 RH	MIC3, ROP8, and SAG1	Effective protection against the parasite achieved an increase in survival time in the immunized mice, especially in the MRS-CaNPs group.	(74)
Inactivated parasite, crude or purified antigens	#	Calcium-Dependent Protein Kinase 7 (CDPK7)	The protein has immunogenic and nonallergenic nature.	(75)
	#	calcium-dependent protein kinase-3 (CDPK3)	It is higher affinity for MHC-binding and CTL epitopes	(76)
	1.5×10^6 RH	Soluble total antigen	STAg increased the release of IL1β. IL18 significantly upregulated after 24 h.	(77)

#: 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 MHCclass1-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 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 *Toxoplasma* 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.

Acknowledgements

We gratefully acknowledge the supporting of the Deputy of Research, Mazandaran University of Medical Sciences, Sari, Iran.

Conflict of interest

None.

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