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A systematic review of *Toxoplasma gondii* antigens to find the best vaccine candidates for immunization

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ABSTRACT

At present, there is not any available accepted vaccine for prevention of *Toxoplasma gondii* (*T. gondii*) in human and animals. We conducted literature search through English (Google Scholar, PubMed, Science Direct, Scopus, EBSCO, ISI Web of Science) scientific paper databases to find the best vaccine candidates against toxoplasmosis among *T. gondii* antigens. Articles with information on infective stage, pathogenicity, immunogenicity and characterization of antigens were selected. We considered that the ideal and significant vaccines should include different antigens and been expressed in all infective stages of the parasite with a high pathogenicity and immunogenicity. Evaluation within this systematic review indicates that MIC 3, 4, 13, ROP 2, RON 5, GRA 1, 6, 8, 14 are expressed in all three infective stages and have pathogenicity and immunogenicity. MIC 5, ROM 4, GRA 2, 4, 15, ROP 5, 16, 17, 38, RON 4, MIC 1, GRA 10, 12, 16, SAG 3 are expressed in only tachyzoites and bradyzoites stages of *T. gondii* with pathogenicity/immunogenicity. Some antigens appeared to be expressed in a single stage (tachyzoites) but have high pathogenicity and induce immune response. They include enolase2 (ENO2), SAG 1, SAG5D, HSP 70, ROM 1, ROM 5, AMA 1, ROP 18, RON2 and GRA 24.

In conclusion, current vaccination against *T. gondii* infection is not satisfactory, and with the increasing number of high-risk individuals, the development of an effective and safe specific vaccine is greatly valuable for toxoplasmosis prevention. This systematic review reveals prepare candidates for immunization studies.

1. Introduction

Toxoplasma gondii, a member of the phylum Apicomplexa, is arguably the most successful protozoan on earth [1]. This intracellular parasite can infect any nucleated cells; however, its only limitation is temperature in poikilothermic animals [2]. Over one billion people are estimated to be infected with this parasite worldwide [3].

T. gondii has a sexual (in feline hosts) and an asexual (in humans and other intermediate hosts) life cycle [4]. Tachyzoites as a rapidly multiplying form, bradyzoites in tissue cysts, and sporozoites in oocysts are three pathogenic forms of this parasite. Humans can be infected with oocyst and tissue-cyst ingestion, as well as congenital infection [5]. Among these pathogens, tachyzoites are responsible for acute phase and clinical manifestations, and bradyzoites account for the chronic phase of the disease.

In an immunocompromised patient, *T. gondii* can lead to a serious disease, and if left untreated, it can cause encephalitis [6]. Toxoplasmosis is considered a serious concern for pregnancy. Congenital *T. gondii* infection in neonates can result in severe neurological birth defects, including mental retardation and blindness [7,8].

Typically, *T. gondii* causes ocular toxoplasmosis in immunocompromised patients; however, the evidence indicates that it can occur in immunocompetent individuals owing to the recurrence of chronic infection in their lifetime [6,9]. At the turn of the 21st century, toxoplasmosis could kill healthy individuals in some areas [2]. Furthermore, some researchers offer that the presence of cysts in the brain can lead to mental disorders [10]. In the United States, *T. gondii* infection is the third important cause of foodborne diseases requiring

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hospitalization [11].

Despite our increasing knowledge about *T. gondii,* we still have a very limited number of treatments to control tachyzoite stage; however, the cyst stage is considered as untouchable chronic infection [12]. For toxoplasmosis treatment, the recommended drugs have side effects, and reactivation may occur at any time [5,13–15]. Nevertheless, there are not any therapies, which can eradicate this organism from the host. On the other hand, immunization against *T. gondii* is another way to control this infection. Therefore, the development of a safe and effective vaccine would be extremely valuable in fighting against *T. gondii* infection [5,16].

The whole human population are at risk of *T. gondii* infection; in this regard, this infection can result in ocular disease in adults. Everyone could benefit from vaccination against this parasite [17]. Moreover, due to the reactivation possibility of latent infection in immunocompromised individuals and primary infection in pregnant women leading to abortion, immunization against toxoplasmosis is necessary.

At present, the only accepted vaccine against toxoplasmosis is "Toxovax", which contains live attenuated S48 strain that controls congenital infection in ewes [5]. Toxovax decreases the abortion rate, but does not eradicate the parasite completely. However, it is expensive and may be changed into a pathogenic form; therefore, it is not appropriate for human use [18]. Furthermore, there is not any licensed vaccine for humans.

For immunization against toxoplasmosis, many vaccination trials, including subunit, protein, DNA, and heterologous and live attenuated vaccinations, have been performed; nonetheless, they only led to partial protection. Although different antigens from *T. gondii* micronems, rhoptries, dense granules organelles, and surface antigens have been tested, the researchers could not find a suitable vaccine for controlling *T. gondii* infection in humans [19–21].

Micronems play an important role in the recognition, attachment, and penetration of parasite to host cells [22], followed by the release of rhoptry contents [23]. At this stage, rhoptry neck proteins (RONs) are secreted into the host cell membrane to help micronemal protein, apical membrane antigens 1 (AMA1), for moving junction [11,24]. Then, bulb of ROPs are discharged into cytosol to interact with host cellular organelles and are important for the biogenesis of the parasitophorous vacuole during the parasite invasion [25]. Finally, dense granules (GRAs), containing GRA proteins, widely modify the parasitophorous vacuole and are thought to contribute to nutrient gain for functioning in intracellular survival and replication [13,25].

Superfamily of surface antigens (SRS) is another *T. gondii* antigens, and its precise role is unclear. *T. gondii* may provide an expressed superfamily of antigenically surface protein adhesins to facilitate entry into host cells, which mediate attachment and stimulate host immune response to regulate the *T. gondii* virulence. Surface antigen glycoprotein (SAG) family is the smallest member of the SRS superfamily [26]. Other *T. gondii* antigens are oocyst wall proteins (OWP) that can show parasite targets for the progress of serologic assays [27].

Many antigens have been identified as vaccine candidates in the last few years; however, the ones with high immunity and long-term protection are restricted. Therefore, the selection of proper antigens is an essential step to design safe and effective vaccines [5]. Immunity induced by *T. gondii* as an intracellular parasite is complex, including both cellular and humoral immune responses [28]. Innate immune cells, such as neutrophils, macrophages, dendritic cells, and natural killer cells, are involved in immune responses against *T. gondii* infection, especially in the production of IL-12 [29]. This cytokine is essential for the regulation of interferon gamma (IFN-γ) production by natural killer and T cells (T-lymphocytes).

Cellular immune response plays a major role in controlling both acute and chronic *T. gondii* infections. T helper cells are responsible for protective immunity through different types of T-lymphocytes (e.g., $CD4^+$, $CD8^+$ T cells) and cytokines, including IL-2, IL-12, IFN-γ, and tumor necrosis factor-α. Other cytokines, including IL-10, IL-4, and IL-5, have a vital function in balancing immune responses [30,31]. Apart from cellular immunity, specific antibodies play an important role in controlling *T. gondii* infection by the inhibition of the attachment of the parasite to the host cell and enhancement of antibody-coated tachyzoites killing through complement-dependent pathway and macrophages [28].

On the other hand, the success of a vaccine depends on several parameters, such as the type of antigen, use of suitable adjuvant in the vaccine formulation, and delivery system, all of which could influence the immune response induced by the vaccine [32]. In the meantime, an appropriate antigen plays a crucial role in the potent induction of long-lasting protection by innate immune cells, namely T- and B-cells, during *T. gondii* infection [33]. Accumulating evidence indicates that the expression of molecules affects *T. gondii* pathogenicity to establish an essential step in the induction of both humoral and cellular immune responses in vaccine development [34].

Furthermore, according to the literature, the use of stage-specific antigens induces strong immune responses and leads to stage-limited protection against toxoplasmosis [35–37]. Hence, significant vaccines should have diverse antigens in all three stages of the parasite with a high pathogenicity and immunogenicity. These recommended indications increase the chance of success in immunization against *T. gondii* infection.

In recent years, studies have focused on finding safe and effective vaccines against *T. gondii*. Accordingly, it is essential to evaluate antigens and identify vaccine candidates that could produce extreme and protective immune responses against toxoplasmosis. Therefore, the goal of the present systematic review was to evaluate the best antigens to be considered as vaccine candidates containing such important characteristics as pathogenicity, immunogenicity, and multi-stage effectiveness. This study was also targeted toward the preparation of comprehensive information for performing more accurate studies in the future.

2. Methods

2.1. Data extraction process

The current study was performed in accordance with the PRISMA statement [38]. To evaluate antigens characterization for the identification of vaccine candidates against toxoplasmosis, English databases, including PubMed, Google Scholar, Science Direct, Scopus, ISI Web of Science, and EBSCO, were browsed from January 1st in 1999 to June 30th in 2017. For this purpose, the present systematic review was carried out using medical subject heading terms and a combination of several keywords including: "*Toxoplasma gondii*", "Toxoplasmosis", "Antigen", "Vaccine", "Pathogenicity", and "Immunogenicity". In order to avoid missing any articles, after database searching, the reference list of the relevant papers was also screened manually.

The abstracts published in congresses or gray literature were not included. The exclusion criteria were: 1) insufficient information, 2) irrelevant abstracts, and 3) unavailability in English. Among articles found with the mentioned strategies, full text papers on vaccines, antigens, and immunization were included. The title and abstract of these papers were assessed by two reviewers (AD, FR). Subsequently, full texts of the relevant articles were carefully examined by the same reviewers. Finally, any disagreements with the other two reviewers (MS, SP) were resolved. The search process resulted in the inclusion of 134 articles.

2.2. Data synthesis

All experimental studies evaluating antigen characterization against *T. gondii* were selected based on the title and abstract, and then they were carefully reviewed to assess the eligibility for inclusion. The

information extracted from each article included stage, pathogenicity, immunogenicity, molecular weight, and characterization of antigens.

In the present study, various criteria were used to evaluate the antigen-immune responses, including cellular immune responses (e.g., induced $CD8^+$ T-cells and lymphocyte proliferation), T cell subsets analysis, humoral immune responses (e.g., total IgG, IgM, IgA, and subclasses of IgG1, IgG2a, and IgG3), cytokines assay (e.g., IFN-γ, IL-2, IL-4, IL-5, IL-6, IL10, and IL12), survival time of immunized mice challenged with parasite, and reduction in the brain cyst burden of immunized mice challenged with *T. gondii*.

All articles were analyses by the two reviewers and reported systematically (AD, FR). A protocol of this systematic review is available in PROSPERO, an international prospective register of systematic reviews (2013) and CRD42017074430 (https://www.crd.york.ac.uk/prospero/).

3. Result

3.1. Analysis of the included literature

In the current systematic review, *T. gondii* antigens were evaluated based on the specific stage, pathogenicity, and immunogenicity characteristics. A total of 134 papers were included in this review. Fig. 1 displays the search process in this systematic review.

In the reviewed articles, various criteria were used to evaluate the pathogenicity and immunogenicity. In pathogenicity, different antigens in tachyzoite, bradyzoite, sporozoite and merozoite stages (Fig. 2) with invasion function or those that were essential factors for invasion were identified by one star (*), and antigens with high virulence or very important factor for invasion were displayed by two stars (**) in tables. Regarding the immunogenicity, the results are presented as one star (*) and two stars (**) in tables. One star (*) means partial immune response/protection and two stars (**) signifies high or strong immune responses/protection against *T. gondii*.

3.2. Toxoplasma gondii antigens

3.2.1. MIC antigens

Evaluation of 18 minimum inhibitory concentrations (MIC) of the antigens (Table 1) indicated that some antigens appeared to be expressed in all stages (i.e., tachyzoites, bradyzoites, and sporozoites) of *T. gondii,* including MIC3, MIC4, and MIC13. Seven MIC antigens, namely MIC1, MIC 2, MIC2AP, MIC5, MIC7, MIC10, and MIC11, are expressed in two infectious stages of *T. gondii* (i.e., tachyzoites and bradyzoites). The MIC10 expression is higher in tachyzoites than in bradyzoites [39]. Other MIC antigens found to be expressed in only one stage. Additionally, pathogenic and immunogenic studies show that MIC1, MIC3, MIC4, and MIC6 had a high pathogenicity, while MIC3, MIC4, MIC5, MIC6, MIC8, and MIC13 demonstrated a high immunogenicity.

3.2.2. AMA antigens

Table 2 presents the results of the included studies regarding apical membrane antigens. It is shown that apical membrane antigen 1 (AMA1) and 2 are just expressed in tachyzoite stage, and that AMA1 has immunogenicity and high pathogenicity. The AMA3 is secreted in sporozoites, and AMA4 is predicted for sporozoite [40].

3.2.3. ROM antigens

Several studies identified that *T. gondii* contains six rhomboids, such as genes including *ROM1-ROM6* (Table 3). These antigens are triggers for micronems activity. *ROM1, ROM4,* and *ROM5* show high pathogenicity and induce strong humoral and cellular immune responses. *ROM 4* is expressed in tachyzoite and bradyzoite [41].

3.2.4. ROP antigens

Table 4 tabulates the details about the investigated rhoptry proteins. ROP1, ROP2, ROP21, and ROP42 are expressed in all three infective stages of *T. gondii*. ROP2 shows high pathogenicity and immunogenicity. Although ROP21 is not essential for tachyzoites, it plays a significant role during chronic infection by the establishment of tissue cysts [42]. In addition, ROP42 is highly expressed in bradyzoites [26].

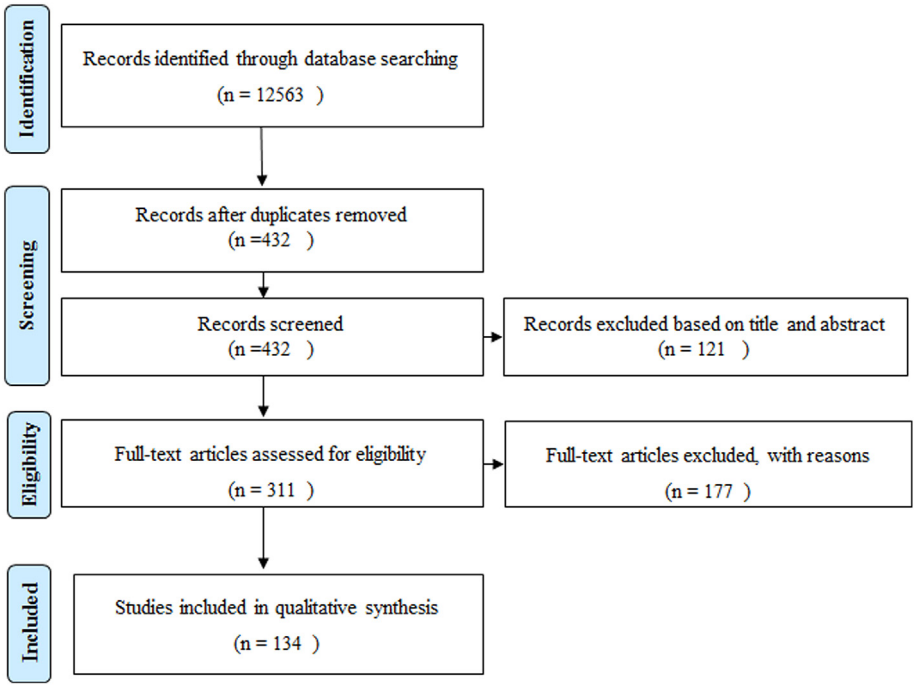

Fig. 1. The PRISMA flow diagram of the search strategy, study selection, and data management procedure of antigens characterization, according to the conditions sets (muti stage, pathogenicity and immunogenicity) to suggestion the best candidates for immunization study.

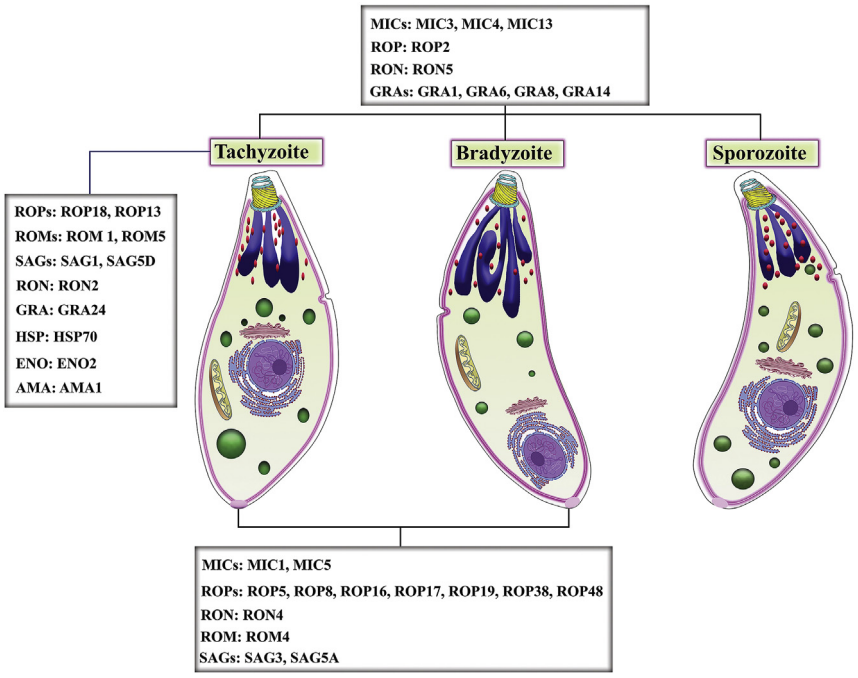

Fig. 2. Summery of important antigens are available in one, two or three stage (tachyzoite, bradyzoite, and sporozoite) of *T. gondii.*

Table 1
Summery of micronem antigens of *Toxoplasma gondii.*

MIC_S	Tachyzoite	Bradyzoite	Sporozoite	Merozoite	Pathogenicity	Immunogenicity	Molcular weight	References
MIC 1	+	+			*	*	60 KD	[34]
MIC 2	+	+			*	*	115 or 120	[89]
MIC 2AP	+	+				*	40 or 43	[89]
MIC 3	+	+	+	+	**	**	90	[16,26,68,69,90–94]
MIC 4	+	+	+	+	*	*	61	[26,34,81,89,95,96]
MIC 5	+	+			*	*	22	[91]
MIC 6	+				*	**	34	[34]
MIC 7	–	+					38	[97]
MIC 8	+				*	*	70	[97–99]
MIC 9	–	+					33	[97]
MIC 10	+	+					18	[39]
MIC 11	+	+			*	*	16	[100,101]
MIC 12				+				[26]
MIC 13	+	+	+		*	**		[27]
MIC 16	+							[26]
MIC 17A				+				[26]
MIC 17B				+				[26]
MIC 17C				+				[26]

In pathogenicity: * means function in invasion or essential factor for invasion and ** means high virulence or very important factor for invasion.
In immunogenicity: * means partial immune response/protection and ** shows high or strong immune responses/protection.

Table 2
Summery of apical membrane antigens of *Toxoplasma gondii.*

AMA	Tachyzoite	Bradyzoite	Sporozoite	Merozoite	Pathogenicity	Imonogenisity	Molcular weight	References
AMA 1	+				*	*	67	[13,26,40,89]
AMA 2	+	_-						[40]
AMA 3	–	–	+					[40,102]
AMA 4	–	–	Predict for sporozoite					[40]

In pathogenicity: * means function in invasion or essential factor for invasion and ** means high virulence or very important factor for invasion.
In immunogenicity: * means partial immune response/protection and ** shows high or strong immune responses/protection.

ROP5, ROP16, and ROP17 are expressed in two infective stages of *T. gondii* (i.e., tachyzoite and bradyzoite) and show high pathogenicity and immunogenicity. In spite of the fact that ROP18 is expressed in only tachyzoite stage, it has a high pathogenicity and induces humoral and cellular immune responses [43].

3.2.5. RON antigens

The study of RON proteins (Table 5) indicated that RON5 is expressed in all three stages and has partial pathogenicity and high immunogenicity. The latter is also critical for RON2 stability and function in invasion [44]. This antigen is recognized to cooperate with AMA1

Table 3
Summery of romboeids antigens of *Toxoplasma gondii*.

	Tachyzoite	Bradyzoite	Sporozoite	Merozoite	Pathogenicity	Immunogenicity	Molcular weight	References
ROM 1	+	–	+		*	**		[103,104]
ROM 2			+					[104]
ROM 3			+					[104]
ROM 4	+	+			*	**		[41,70,104–106]
ROM 5	+				*	**		[70,104,107]
ROM 6						is predicted to be a mitochondrial PARL-like ROM		[104]

In pathogenicity: * means function in invasion or essential factor for invasion and ** means high virulence or very important factor for invasion.
In immunogenicity: * means partial immune response/protection and ** shows high or strong immune responses/protection.

during moving junction and host cell invasion [45]. RON4 is expressed in the merozoite; nonetheless, it is down-regulated, compared to the tachyzoite and bradyzoite [46].

3.2.6. GRA antigens

Table 6 lists the evaluated GRA antigens. The GRA1, GRA5, GRA6, GRA8, and GRA14 are expressed in all three stages of *T. gondii*. GRA1 is expressed by tachyzoites, sporozoites, and bradyzoites [47]. Expression of GRA8 is higher in oocysts than in bradyzoites; however, it is not different from that in tachyzoite [27]. Furthermore, GRA14 expression is higher in oocysts than in tachyzoites and bradyzoites. GRA23 is low antigenic during acute infection and high antigenic during chronic infection [48]. Antibodies against GRA1 and GRA5 are the markers of chronic infection, and GRA6, GRA7, and GRA8 are reported as the markers of acute infection. GRA4, GRA10, GRA12, and GRA15 are expressed in both tachyzoites and bradyzoites, and have high pathogenicity and strong immunogenicity characteristics.

3.2.7. SRS & SAG antigens

Surface proteins were significantly expressed in merozoites than in tachyzoites. In the SAG family genes, *SAG1* is only observed in tachyzoite stage and is highly conserved among *T. gondii* strains. It shows high pathogenicity and induces both humoral and cellular immune responses. *SAG 2A* may be used as an indicator for the acute phase of *T. gondii* infection [49], *SAG 2C, SAG D, SAG X*, and *SAG Y* are important for the persistence of cyst in the brain [50,51]. They might be considered as the indicators of cyst maturation.

SAG3 is observed in both tachyzoite and bradyzoite and has a good pathogenicity and immunogenicity. Similar to *SAG1, SAG5* antigens may have strong immunogenicity [52], and has been proved to have a

Table 4
Summery of rhoptry antigens of *Toxoplasma gondii*.

ROP	Tachyzoite	Bradyzoite	Sporozoite	Merozoite	Pathogenicity	Immunogenicity	Molcular weight	References
ROP 1	+	+	+			*	60. 5or 68	[37,108,109]
ROP 2 (PK)	+	+	+		*	**	55 or 66	[58,64,110]
ROP 3	+				*		59 or 63	[111]
ROP 4 (PK)	+	+			*		60	[111,112]
ROP 5 (PK)	+	+			**	**	60	[46,62,113,114]
ROP 6 (PK)	+	+			*		42	[115]
ROP 7 (PK)	+	+			*		57	[111,116]
ROP 8	+	+				**		[117]
ROP 9 (P 36)	+	–	+			*		[118,119]
ROP 13	+					**		[120]
ROP 16 (PK)	+	+			**	*	52	[46,121,122]
ROP 17 (PK)	+	+			**	*		[123]
ROP 18 (PK)	+				**	*		[43,46,113,114]
ROP 19 (PK)	+	+				**		[124,125]
ROP 20	+							[126]
ROP 21	+	+	+	+				[42,46]
ROP 23	+							[26]
ROP 26	+							[127]
ROP 27	–	+	+					[42]
ROP 28	+		+					[42,127]
ROP 29	+	+						[127]
ROP 30	+							[42]
ROP 32				+				[46]
ROP 33				+				[46]
ROP 35	+	+						[46]
ROP 36				+				[46]
ROP 38)ROP 2L5)	+	+			*	**	66	[128]
ROP 39	+							[26]
ROP 40	+							[26]
ROP 41	+							[26,127]
ROP 42	+	+	+	+				[26,27]
ROP 43				+				[26]
ROP 44	+							[26]
ROP 46	+			+				[26]
ROP 48	+	+				*	39	[129]

In pathogenicity: * means function in invasion or essential factor for invasion and ** means high virulence or very important factor for invasion.
In immunogenicity: * means partial immune response/protection and ** shows high or strong immune responses/protection.

Table 5
Summery of rhoptry neck Proteins antigens of *Toxoplasma gondii*.

	Tachyzoite	Bradyzoite	Sporozoite	Merozoite	Pathogenicity	Immunogenicity	Molcular weight	References
RON 1	+						127	[130]
RON 2	+	–	+	+	*	*	155	[26,40,131]
RON 2-L1			Predict for sporozoite					[40]
RON 2-L2 (Sporo RON)			+					[40]
RON 3	+						223	[130]
RON 4	+	+			*	*	107	[130–132]
RON 4-L1	+	+		+				[27]
RON 5	+	+	+		*	**	179	[59]
RON 8	+	–		+	**			[26]
RON 9	+							[133]
RON 10	+							[133]

In pathogenicity: * means function in invasion or essential factor for invasion and ** means high virulence or very important factor for invasion.
In immunogenicity: * means partial immune response/protection and ** shows high or strong immune responses/protection.

Table 6
Summery of dense granule antigens of *Toxoplasma gondii*.

GRA	Tachyzoite	Bradyzoite	Sporozoite	Merozoite	Pathogenicity	Immunogenicity	Molcular weight	References
GRA1 (P 24)	+	+	+		*	**	24	[47,109,134]
GRA2	+	+				*	28.5	[135,136]
GRA3	+	? +			*		30	[46,137]
GRA4	+	+				*	40	[71,134,138–140]
GRA5 (P 21) (16)	+	+	+		*	*	21	[69]
GRA6	+	+	+		*	*	32	[46,141]
GRA7 (P 29)	+	+		+		*	29	[46,134,142,143]
GRA8	+	+	+			*	38	[27,46,144]
GRA9 (B 10)	+	+					41	[145]
GRA10	+	+			*	*	36	[146]
GRA 11A				+				[26]
GRA 11B				+				[26]
GRA12	+	+		+	*	*		[46,147]
GRA14	+	+	+		*	*	47	[27,148,149]
GRA15	+	+			*	*		[46]
GRA16	+	+						[26,48]
GRA19	+							[48]
GRA20	+							[48]
GRA21	+							[48,119]
GRA22	+							[145]
GRA23	+	+						[48]
GRA24	+				*	*		[46]

In pathogenicity: * means function in invasion or essential factor for invasion and ** means high virulence or very important factor for invasion.
In immunogenicity: * means partial immune response/protection and ** shows high or strong immune responses/protection.

better antigenic effect than *SAG1*. *SAG 5A* is not expressed in RH strain tachyzoites. SAG 5B and SAG C proteins are expressed in two stages of tachyzoites and bradyzoites. *SAG 5D* protein is expressed only in tachyzoite and is highly pathogenic and immunogenic [53]. *SAG 5E* is a transcribed pseudogene. Evaluation of SRS and SAG antigens are shown in Table 7.

3.2.8. Oocyst wall protein

Oocyst wall protein (OWP) (Table 8) are just expressed in the sporozoites infective stage of *T. gondii*. CCP5A is another sporozoite-specific wall protein that can be used in diagnostic and epidemiological studies as a new marker to identify the source of infection in animals and humans [54].

3.2.9. Other antigens

Details about other investigated antigens are illustrated in Table 9. Matrix antigen 1 (MAG1), a special marker of acute infection, may be useful in the preliminary detection of recent *T. gondii* infection. *ENO2,* expressed in tachyzoite and merozoite, plays an important role in metabolism, and has high pathogenicity and immunogenicity [55]. HSP30, a small heat shock protein, is specific for bradyzoite stage that stimulates the differentiation of tachyzoites to bradyzoites [56]. HSP70, a tachyzoite-specific virulent molecule, is another *T. gondii* heat shock protein antigen, which rapidly increases just before the death of the host [57]. HSP70 is an antigen with pathogenicity and immunogenicity characteristics.

3.2.10. The bets antigen candidates for Toxoplasma gondii immunization

In regard to evaluation of pathogenicity and immunogenicity of different antigens in tachyzoite, bradyzoite, sporozoite and merozoite of this protozoan, the best antigen candidates for immunization studies have been shown in Table 10.

4. Discussion

This systematic review aimed to characterize *T. gondii* antigens to suggest the best vaccine candidates, considering their stage specificity, pathogenicity, immunogenicity, and molecular weight. Currently, there is no licensed vaccine available for humans; therefore, the development of a new, safe, and effective vaccine with multi mechanisms of action is a global priority [5].

An ideal vaccine must have different antigens in all three infected stages of *T. gondii*, high pathogenicity, and capacity to induce strong immune responses. However, there is currently no available vaccine

Table 7
Summery of SRS & SAG antigens of *Toxoplasma gondii*.

SRS& SAG	Tachyzoite	Bradyzoite	Sporozoite	Merozoite	Pathogenicity	Immunogenicity	Molcular weight	References
SRS 28 (sporo SAG)			+					[47,150]
SRS 8 (SRS19B)								[27]
SRS 9	–	+					43	[51]
SRS 27B				+				[26]
SRS 3 (SRS 51)	+							[27]
SRS 38A								[27]
SRS 16	–	+						[27]
SRS 16B	+							[26]
SRS 20A	+							[26]
SRS 22A				+				[26]
SRS 22B				+				[26]
SRS 29A	+							[26]
SRS 29B	+							[26]
SRS 29C	+							[26]
SRS 34A	+							[26]
SRS 35A	+							[26]
SRS 35B	+							[26]
SRS 36C	+							[26]
SRS 36D	+							[26]
SRS 36E	+							[26]
SRS 44	+							[26]
SRS 51	+							[26]
SRS 52A								[27]
SRS 67	+							[26]
SRS 42								[27]
SRS 20A	+							[26]
SRS 2 (SRS 29C)	+							[46]
BSR 4	–	+		+			36	[46]
SRS 1	+							[27]
SAG 1(P30) (SRS 29B)	+	–			**	**	30	[73,79,95,134,151–154]
SAG 2A (SRS 34A)	+	–						[49,50]
SAG 2B	+							[50]
SAG 2C (SRS 49D)	–	+						[46,50,51]
SAG 2D (SRS 49C)	–	+				.		[46,50,51,155]
SAG 2X (SRS 49B)	–	+						[50,51]
SAG 2Y (SRS 49A)	–	+						[50,51]
SAG 3 (SRS 57)	+	+			*	*	43	[50,134,156–158]
SAG 4A (P 18)	_-	+					18	[27]
SAG 4.2 (SAG 4B)		+						[27]
SAG 5A	+	+				**		[159]
SAG 5B	+	+						[63,159,160]
SAG 5C	+	+						[63,159,160]
SAG 5D	+	–			**	**		[53,63]
SAG 5E	Is a transcribed pseudogene							[63]

In pathogenicity: * means function in invasion or essential factor for invasion and ** means high virulence or very important factor for invasion.
In immunogenicity: * means partial immune response/protection and ** shows high or strong immune responses/protection.

fulfilling these criteria. Consequently, the results of this systematic review can be helpful in setting the ground for performing more accurate studies in the future with the aim of developing successfull vaccines against *T. gondii* by the selection of more effective antigens.

4.1. Stage-specific antigens of Toxoplasma gondii

T. gondii possesses an intricate life cycle with varied life stages [4]. Base on the results of the reviewed articles regarding toxoplasmosis vaccination, multi-stage antigens result in more protection [58,59]. A number of these parasite antigens were secreted in all tachyzoite, bradyzoite, and sporozoite stages; therefore, they were considered as appropriate candidates for vaccine studies [2,27,58].

Accordingly, among the various groups of antigens, MIC 3, MIC4, MIC13, RON5, ROP2, GRA1, GRA6, GRA8, and GRA14 were reported to be available in all three stages. Some antigens are expressed only in tachyzoites and bradyzoites stages of *T. gondii*. These antigens include MIC (MIC1, MIC5, MIC10, MIC11), RON4, ROM4, ROP (ROP5, ROP8,

Table 8
Summery of oocyst wall protein antigens of *Toxoplasma gondii*.

OWP	Tachyzoite	Bradyzoite	Sporozoite	Merozoite	Pathogenisity	Immunogenicity	Molcular weight	References
OWP 1			+					[27]
OWP 2			+					[27]
OWP 3			+					[27]
OWP 4			+					[27]
OWP 5			+					[27]
OWP 7			+					[27]
CCP5A			+					[56]

Table 9
Summery of some other antigens of *Toxoplasma gondii*.

	Tachyzoite	Bradyzoite	Sporozoite	Merozoite	Pathogenicity	Immunogenicity	Molcular weight	References
MAG 1	+	+					65	[161,162]
BAG 1	–	+					30	[46,163]
CWA	–	+					115	[163]
ENO 1	–	+						[76,163]
ENO 2	+	–		+	**	**		[46,55,75,163]
LDH 1	+	–		+				[46,163]
LDH 2	–	+						[163]
HSP 20	+	+				*		[164]
HSP 30	–	+				*		[56]
HSP 60	+	+						[165]
HSP 70	+				*	**		[56,57,166,167]
HSP 90	+	+					82	[165,168]

In pathogenicity: * means function in invasion or essential factor for invasion and ** means high virulence or very important factor for invasion.
In immunogenicity: * means partial immune response/protection and ** shows high or strong immune responses/protection.

Table 10
Suggestion antigen candidates for *Toxoplasma gondii* vaccine.

Group	Antigens	Tachyzoite	Bradyzoite	Sporozoite	Pathogenicity	Immunogenicity
A						
	MIC 3	+	+	+	*	**
	MIC 4	+	+	+?	*	**
	MIC 13	+	+	+	*	**
	ROP 2	+	+	+	*	**
	RON 5	+	+	+	*	**
	GRA1 (P 24)	+	+	+	*	**
	GRA 6	+	+	+	*	*
	GRA8	+	+	+	*	*
	GRA 14	+	+	+	*	*
B						
	MIC 5	+	+		*	**
	ROM 4	+	+		*	**
	GRA 2	+	+		*	**
	GRA 4	+	+		*	**
	GRA 15	+	+		*	**
	ROP 5	+	+		**	*
	ROP 16	+	+		**	*
	ROP 17	+	+		**	*
	ROP 38	+	+		*	**
	)ROP 2L5)					
	ROP 8	+	+			**
	ROP 19	+	+			**
	ROP 48	+	+			**
	SAG 5A	+	+			**
	RON 4	+	+		*	*
	MIC 1	+	+		*	*
	GRA 10	+	+		*	*
	GRA 12	+	+		*	*
	GRA16	+	+		*	*
	SAG 3	+	+		*	*
C						
	ENO 2	+			**	**
	SAG 1	+			**	**
	SAG 5D	+			**	**
	HSP 70	+			*	**
	ROM 1	+		+	*	**
	ROM 5	+			*	**
	AMA 1	+			*	*
	ROP 18	+			**	*
	ROP 13	+				**
	RON 2	+		+	*	*
	GRA 24	+			*	*

GROUP A: Antigens seen in tachyzoite, bradyzoite, sporozoite and with high pathogenicity & immunogenicity.
GROUP B: Antigens seen in tachyzoite, bradyzoite and with high pathogenicity & immunogenicity.
GROUP C: Antigens seen in tachyzoite and/or sporozoite and with high pathogenicity & immunogenicity.
In pathogenicity: * means function in invasion or essential factor for invasion and ** means high virulence or very important factor for invasion
In immunogenicity: * means partial immune response/ protection and ** shows high or strong immune responses/ protection

ROP16, ROP17, ROP19, ROP38, ROP48), GRA (GRA2, GRA4, GRA10, GRA12, GRA15, GRA16), and ENO2. The use of multi-stage antigens can be very important in producing a vaccine, regarding that they can contribute to deal with the initial and recurrent infections and have an important role in removing the bradyzoites released from tissue cysts.

Furthermore, many investigations indicated that cocktailed vaccines could induce significantly higher immunogenicity than single gene vaccines [41,60,61]. Therefore, the utilization of multi-stage antigens or cocktailed vaccines can be more effective.

4.2. Pathogenicity of Taxoplasma gondii

Many investigations have revealed that *T. gondii* antigens with high pathogenicity are more efficient to stimulate the immune response and could be introduced as suitable immunization candidates for toxoplasmosis prophylaxis [1,62–64]. In this review, we determined that MIC3, ROM1, ROM4, ROM5, ROP2, ROP5, ROP16, ROP18, GRA6, GRA10, SAG1, SAG 5D, and ENO2 antigens have shown higher pathogenicity than other antigens.

Invasion is an essential event in the life cycle and pathogenicity of *T. gondii* and has received particularly intense attention. Cell invasion is a wrapped process that is not completely recognized in spite of extensive studies [2]. This complex process essentially involves both surface antigens and apical secretory organelles of parasite, related to exclusive gliding motility system [2,65]. Based on the evidence, the consecutive secretions of apical secretory organelles of *T. gondii* are definitive events for the invasion and establishment of this parasite in the host cells [11,13].

Micronems commonly discharge adhesive antigens that are necessary for motility and invasion [13]. Among micronemal antigens, MIC3 (90 KD) is a strong adhesion of *T. gondii* [66], and several studies have shown that this antigen has a high pathogenicity. Consequently, it seems to be a suitable candidate for immunization studies against toxoplasmosis [67–69]. Rhomboids are a family of serine proteases that are reported to have an important role in the regulation of growth factor and parasite invasion [41]. Among rhomboids antigens, ROM1, ROM4, and ROM5 show high pathogenicity and could be good candidates for immunization against *T. gondii* infection [70,71].

Rhoptries are other secretory organelles that affect the invasion of parasite and time of the ROP_S antigens discharge; therefore, they target the surface or parasitophorous vacuole of the host cells [1,23]. Various studies indicate that some rhoptry antigens, such as ROP2, ROP5, ROP16, and ROP18, have a high pathogenicity as major virulence components [2,6,72]. Considering the role of rhoptry antigens in virulence and satisfactory immunogenicity, they could be promising vaccine candidates against *T. gondii* [1,11].

T. gondii GRA antigens are involved in parasitic survival, virulence, and replication [25]. Among these antigens, GRA6 and GRA10 have shown a high pathogenicity, and GRA1, GRA2, GRA3 are important for virulence. Therefore, these antigens could be effective candidates for immunization against toxoplasmosis [2,35]. Surface proteins of *T. gondii* encode SAG family genes [73]. In this family, *SAG1* and *SAG 5D* with a high pathogenicity could be the best candidates against *T. gondii* infection. In addition, *SAG 5D* gene was proved to have better antigenicity than *SAG1* [52].

Enolase is an extremely preserved protein that is frequently vital to cellular function and found in numerous organisms, including *T. gondii* [74]. Considerable pieces of evidence has revealed that enolase promotes pathogen host interactions and contributes to infection and pathogenesis [55]. Two isoforms of enolase are encoded in *T. gondii* gene. Enolase 1 is secreted particularly in bradyzoite, while enolase 2 is secreted in tachyzoite [75,76]. Enolase 2, an important antigen of *T. gondii*, is a surface exposed protein with strong pathogenicity and immunogenicity [55]. Consequently, this antigen may serve as a vaccine candidate against toxoplasmosis.

4.3. Immunogenicity of Taxoplasma gondii

The evidence showed that during the natural infection with *T. gondii*, host immune system responds to all parasite antigens and is capable of inducing complete protective immunity. However, infection with *T. gondii* or use of live attenuated vaccine of this parasite has severe side effects. Toxovax as an attenuated strain of *T. gondii* vaccine has been successfully employed for sheep and goats; however, this vaccine is not suitable for humans due to the risk of reversing pathogenic strain and its side effects [5,14,19].

Therefore, it is necessary to use safe, effective, and well-tolerated antigens that are able to eliminate the parasite and subsequently prevent cyst formation and maternal-fetal transmission. The aforementioned evidence is indicative of the high success likelihood of developing a vaccine against toxoplasmosis. Recently, remarkable advances have been reported in the evaluation of vaccine candidates that can stimulate strong immunity against this infection. In *T. gondii*, surface antigens and excretory-secretory antigen (ESA) have been known as possible vaccine candidates. In an animal study, these antigens demonstrated an important protective role against *T. gondii* strain by increasing survival time or decreasing cysts loads in the brain [32].

Several studies have investigated SAG family antigens, including SAG1 (30 kDa), SAG2 (22 kDa), and SAG3 (43 kDa). Among these antigens, SAG1 (P30) as a predominant vaccine candidate affects the binding of tachyzoites to host receptors and invasion into cells [77]. Based on the reviewed studies, the highest protection rate for SAG1 against acute infection was obtained as 90%, and no brain cysts were observed in outbred mice after the administration of carrier saponin Quil A [32]. Furthermore, in another study, SAG1 was reported to result in the survival rate of 80–100% for RH-infected animals immunized with the p1tpA-SAG1, whereas the control group displayed a survival rate of 20% [78].

It is well established that ESA released from secretory organelles, such as micronemes, rhoptries, and dense granules, are considered as potential vaccine candidates to induce humoral and cellular immune responses [79]. ESA are involved in recognition and attachment to the host cell membrane, immune escape of the parasite, intracellular survival and proliferation of parasitophorous vacuole, and pathogenesis [68,80]. This study has revealed that the major components of ESA, such as MIC1, MIC3, MIC4, MIC5, MIC6, MIC8, and MIC13 from micronems, GRA1, GRA2, GRA4, GRA7, GRA10, GRA12, and GRA14 from dense GRA, ROP2, as well as ROP5, ROP16, ROP17, and ROP18 from rhoptries are suitable to be used as vaccine candidates against toxoplasmosis.

Studies in this field have given fruitful results, reporting that MIC1 and MIC4 proteins reduced the number of brain cysts by 68% after challenging with ME49 strain of *T. gondii* in C57BL/6 mice and produced 80% survival rate [81]. One of the most important antigens as a vaccine candidate is MIC3 that is expressed in all three stages of parasite life and is a significant protein for invasion into the host cell [67]. Recently, ROP18 has been confirmed as a candidate for *T. gondii* vaccine with a high immunogenicity in different animal models [82]. In addition, there is substantial evidence showing that ROP2 antigen could well stimulate human T-cell clones for regulating IFN− γ-mediated host resistance [83].

To the best of our knowledge, vaccine delivery system is a factor that can affect the success of vaccination. In different studies, several routes of administration to animal models, including intramuscular, subcutaneous, intraperitoneal, intranasal, and oral, were used. The selection of vaccination route is influenced by the type of immune response you need to induce (e.g., humoral, cellular, or both), vaccination (subunit or DNA), and adjuvant [84]. Intramuscular injection is used to deliver a vaccine deep into the muscles, which allows the vaccine to be absorbed into the bloodstream quickly [85]. Subcutaneous injection is administered into the skin and probably increases the exposure rate of vaccine by the antigen-presenting cells and possibly evokes better

immune responses. Immunization via intranasal route is an effective way for a vaccine to induce mucosal and systemic immune responses [86,87].

Recently, most researchers have focused on the parasite cocktail antigens. This form of vaccine is a combination of different antigens derived from several stages of parasite life cycle. It is noteworthy that the induction of immunogenicity by a cocktail antigen produces a strong immunity against acute and/or chronic phase of toxoplasmosis, which is one of the most important strategies for the development of vaccine [16]. For instance, the use of a cocktail DNA vaccine combining SAG1 with ROP2 caused high-level protection against acute toxoplasmosis in BALB/c mice [88], but not for the single-gene DNA vaccines. In another study, a vaccine cocktail SAG1 + MIC3 induced significantly prolonged protection, compared with a single-gene vaccine [16].

Moreover, the selection of appropriate adjuvant is more important for enhancing the immunogenicity of the vaccine antigenic components than the other parameters. Therefore, it is suggested to compare the efficacy of different adjuvants with candidate antigens to elicit an immune response using standardized protocols in animal models [32]. There are various factors to help antigens promote the immune response, such as adjuvant, routes of vaccine administration, antigen doses, and method of antigen preparation. Consequently, to find a suitable candidate antigen, it is necessary to select all cases with accuracy.

5. Conclusion

As current vaccination against *T. gondii* infection is not satisfactory, and the number of high-risk individuals is increasing, the development of an effective and safe specific vaccine is of paramount significance for toxoplasmosis protection. To improve the disease control, vaccine can be used as an immunotherapeutic strategy, which stimulates potent immune response against *T. gondii* infection or passive immunization in toxoplasmosis reactivation. This systematic review, according to the conditions (i.e., multi-stage antigens, pathogenicity, and immunogenicity), introduced the best candidates for immunization studies.

Antigens that are expressed in three stages with high pathogenicity and immunogenicity could be suitable candidates for immunization studies, such as MIC3, MIC4, MIC13, ROP2, RON5, GRA1, GRA6, GRA8, and GRA14. Some antigens that are expressed in two infectious stages, namely tachyzoite and bradyzoite, (e.g., MIC1, MIC5, ROP5, ROP8, ROP16, ROP17, ROP19, ROP38, ROP48, RON4, ROM4, GRA2, GRA4, GRA10, GRA12, GRA15, GRA16, SAG3, SAG 5A), or in one stage (e.g., ENO2, SAG1, SAG 5D, HSP70, ROM1, ROM5, AMA1, ROP18, ROP13, RON2, and GRA24) with the highest pathogenicity and strong immunogenicity, can be used in cocktailed vaccines to obtain better results for immunization.

5.1. Future perspectives

Future studies should be focused on cocktailed vaccines to devise effective and safe immunization against *T. gondii* infection. Many *T. gondii* antigens are important for the pathogenicity and immunogenicity of this parasite. Therefore, future investigations should focus on antigen quantity and identify potential candidates against toxoplasmosis. Further advanced studies are also required to recognize recombinant vaccine, DNA vaccine action, and selection of the best adjuvants. No study has been performed for immunization through oocyst wall proteins. Furthermore, it is essential to perform further investigations on the use of these antigens to suggest vaccine candidates against *T. gondii* in cats. A number of the antigens reviewed in this paper were found to be suitable for finding an effective vaccine. Moreover, the future in silico and bioinformatic investigations can identify new potential vaccine candidates against toxoplasmosis.

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Appendix A. Supplementary data

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