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Toxoplasmosis in rodents: A systematic review and meta-analysis in Iran

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ABSTRACT

During recent years, implication of rodents in the epidemiology of *Toxoplasma gondii* is overlooked in Iran; thus, we performed a systematic review and meta-analysis to evaluate the prevalence of toxoplasmosis in rodents of Iran. For this purpose, following the general methodology recommended for systematic reviews and meta-analysis, 5 English and 3 Persian databases were explored from 1 January 2000 till 10 September 2016 using related keywords. Finally, 9 out of 291 citations were met to be included in this study. Due to significant heterogeneity, the random-effects model was conducted (I^2 = 93.55%). During the years, 661 rodents were trapped, and 121 of them were identified positive for *T. gondii* 15% (95% CI = 5–27). Moreover, overall prevalence using direct microscopic examination (1/230), PCR-based techniques (41/246) and serological tests (83/437) was obtained 0.1% (95% CI = 0.0–1.5), 18% (95% CI = 4–39) and 15% (95% CI = 3–33), respectively. Our study revealed the prevalence of toxoplasmosis in rodents is remarkable. Considering this fact, they play a key role in the life cycle of *T. gondii* and should not be neglected. Further surveys is needed to better recognize the role of various rodent species in distribution of toxoplasmosis.

Contents

Introduction

Toxoplasma gondii is an obligate intracellular member of phylum *Apicomplexa* which is the most prevalent parasitic infection in humans and many warm blooded animal species that involves one third of the world's population specifically in developing countries [1,2]. Many years have passed since the discovery of this protozoan

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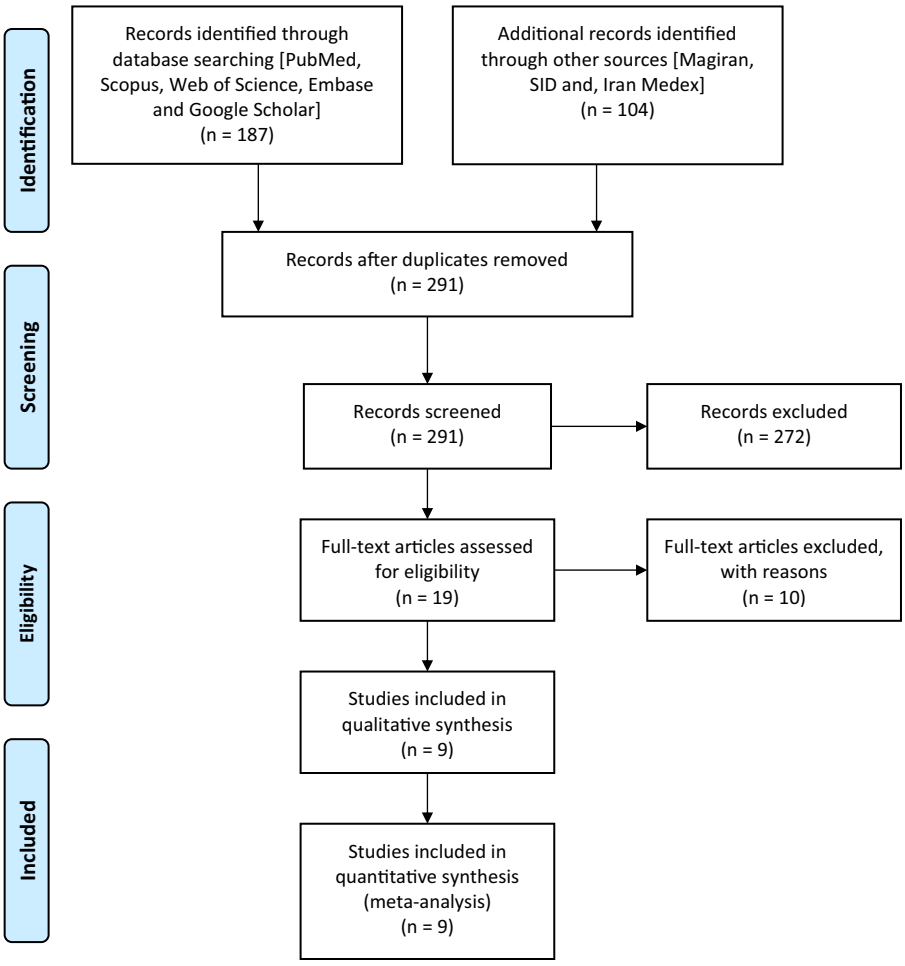

Fig. 1. PRISMA flowchart describing the study design process.

by Nicolle and Manceaux in the smears of liver and spleen of a North African rodent named *Ctenodactylus gondii* in 1908 [3]. *T. gondii* to complete asexual and sexual replication stages in life cycle, needs both intermediate and definitive hosts (Felidae family as definitive host) [1,4]. Toxoplasmosis cases are more dominant in tropical and sub-tropical regions and it has been shown that global warming dilemma has a crucial role in disease distribution [2]. *T. gondii* is mainly transmitted through ingestion of undercooked meat contaminated by cysts or drinking water contaminated by oocyst and congenitally [5–9]. However, transmission via organ transplantation and blood transfusion is less common [10–13]. *T. gondii* in immunocompetent persons is mostly asymptomatic, although in immunocompromised cases such as transplant recipients, HIV^+ individuals, cancer patients, etc. early acute phase of toxoplasmosis would occur or reactivate and causes some complications with poor prognosis including: encephalitis, brain abscess, myocarditis, and chorioretinitis [14,15]. Moreover, chronic toxoplasmosis is strongly correlated to neurodegenerative disorders and autoimmune diseases [16–19]. Previous papers were indicated high morbidity and prevalence of this opportunistic protozoa in variety of hosts in Iran [4,8,11,12,14,20–23].

Rodents are small mammals with short period of maturation and high adaptations ability in terms of biological and morphological features to survive in arboreal, aquatic and terrestrial environments [24]. Role of rodents in spread and transfer of infectious agents is clearly evident. They considered as carrier or reservoir for several infectious agents such as bacteria, virus, fungi and parasites, and transmitted the pathogens through different routes. For instance, they acts as carrier or reservoir for parasitic disease such as: toxoplasmosis, babesiosis, neosporosis, cryptosporidiosis, leishmaniasis, giardisis, amoebic dysentery, chagas disease; Trematoda (schistosomiasis, echinostomiasis, brachylaimiasis, alariasis and human fasciolosis), Cestoda (taeniasis, echinococcosis and rodentolepiasis) and Nematoda (trichinosis, capillariasis, angiostrongylosis, toxoscariasis, aelurostrongylosis and baylisascariasis) [24–27].

Several species of rodents have been identified throughout Iran that probably play a potential role in establishment and maintenance of *T. gondii* life cycle in rural and urban regions [25]. Due to lack of a comprehensive report about the prevalence of *T. gondii* in rodents of Iran, this study was aimed to systematically assess the rate of *Toxoplasma* infection in this overlooked hosts.

Methods

To evaluate the prevalence of toxoplasmosis in rodents of Iran, five English databases (PubMed, Scopus, Web of Sciences, Embase, and Google Scholar) and three Persian databases (Magiran, Scientific Information Database, and Iran Medex) were browsed from 1 January 2000 to 10 September 2016. For this purpose, the present systematic review was done using medical subject headings (MeSH) terms and a combination of several keywords including: "*Toxoplasma*"; "*T. gondii*"; "Toxoplasmosis"; "Prevalence"; "Epidemiology"; "Rodents"; "Rodentia"; "Rat"; "Mouse"; and "Iran". After database searching; the reference list of relevant articles were screened manually. Initially all relevant papers were

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Table 1
Baseline characteristics of included studies.

No.	Province	Year of publication	Sample size (n)	Positive by. . . n (%)			Total prevalence n (%)	More details	Main findings or suggestions	Ref.
				Direct microscopy examination	PCR	Serology				
1.	Khuzestan	2001	90	–	–	0 (0)	0 (0)	A total of 90 rodents including: 14 *M. musculus*, 4 *R. rattus* and 72 *R. norvegicus* were trapped. IFA method was utilized.	–	[37]
2.	Gilan	2002	100	0 (0)	–	24 (24)	24 (24)	A total of 100 rodents including: 7 *R. rattus* and 93 *R. norvegicus* were trapped. Dye test was performed.	24 cases (23 of 93 *R. norvegicus* and 1 of 7 *R. ruttus*) were positive.	[36]
3.	Tehran	2011	68	–	–	25 (36.7)	25 (36.7)	ELISA method was employed.	Maximum number of infected rats were found in the South and Central parts of Tehran with 11.7 percent and with minimum of 1.47 percent in the West of Tehran.	[35]
4.	West Azerbaijan	2012	54	–	12 (22.2)	–	12 (22.2)	529 bp—B1 gene	PCR-RFLP showed type 1 *T. gondii*. The results indicated that the same strain of *T. gondii* can infect human and mouse in surveyed region.	[32]
5.	Tehran	2012	40	–	20 (50)	–	20 (50)	529 bp SDKF (5′-TTAGGTCTACGTGACACAGACGTC-3′) SDKR (5′-CTGCAGACACAGTGCATCTGGATT-3′)	The sequences of the isolates from the present study are accessible under GenBank accession nos. HM569597–HM569603. It can be stated that rats play an important role in the preservation of the *Toxoplasma* life cycle in Tehran.	[33]
6.	Khuzestan	2012	127	–	–	31 (24.4)	31 (24.4)	ICA was performed to detect serum antibodies against *T. gondii*.	*T. gondii* prevalence was higher among female rats (24.66%) than among male rats (24.07%). The prevalence was high for rats captured in the summer season (34.48%) and for rats captured from than East region (36.36%), but no significant differences in the prevalence of infection were identified between genders, seasons, or regions of capture ($P > 0.05$). Our study showed that the seroprevalence of *T. gondii* was relatively high (24.41%) among wild rats in the Ahvaz district of Iran. The high prevalence of *T. gondii* infection in rodents may be of epidemiological importance as infected rodents are a potential route for *T. gondii* transmission to Felidae via ingestion of tissue cysts.	[34]

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Table 1 (*Continued*)

No.	Province	Year of publication	Sample size (n)	Positive by. . . n (%)			Total prevalence n (%)	More details	Main findings or suggestions	Ref.
				Direct microscopy examination	PCR	Serology				
7.	Khuzestan	2014	100	1 (1)	6 (6)	–	6 (6)	A total of 100 rodents (73 *R. norvegicus*, 21 *R. rattus*, and 6 *M. musculus*) were collected. GRA6 PCR F: 5′-GTAGCGTGCTTGTTGGCGAC-3′ R: 5′-ACAAGACATAGAGTGCCCC-3′	PCR showed 6/100 (6%) positive samples (4 from *R. norvegicus* and 2 from *R. rattus*), being all from brain tissues. No positive result was detected from *M. musculus* species. The results were submitted to DDBJ/Genbank at accession nos.: AB743592–AB743597. This finding indicated that *R. norvegicus* and *R. rattus* could be a significant source of *T. gondii* infection for stray cats in the urban region.	[31]
8.	Kohgiluyeh and Boyer-Ahmad	2016	52	–	3 (5.7)	3 (5.7)	3 (5.7)	A total of 52 rodents were captured during the course of this study, including 25 *Meriones*, 15 *Rattus*, 10 *Apodemus*, 1 *Calomyscus*, and 1 (1.9%) *Arvicola*. MAT was performed on rodent sera samples to assess anti-*T. gondii* antibodies. MAT titer of 1:40 or higher were considered as positive. 529 bp gene F: CAG GGA GGA AGA CGA AAG TTG R: CAG ACA CAG TGC ATC TGG ATT	Rodents infected with *T. gondii* were from *Apodemus*, *Meriones*, and *Calomyscus* genus. this is the first report of molecular detection of *T. gondii* infection in *Calomyscus* from Iran. *T. gondii* is common in rodents and these animals can behave as natural reservoir for this protozoa. Evaluation of *T. gondii* infection in rodents, as the main pray for cat, with regards to the role of cat in spreading of *T. gondii* oocyst in the nvironment, is important.	[29]
9.	Khuzestan	2016	30	0 (0)	–	–	0 (0)	Three species of rodents were recognized including: 4 *M. musculus*, 20 *R. norvegicus* and 6 *T. indica*. The brains were smashed and centrifuged and stained by Giemsa for detection of bradyzoite of *Toxoplasma* tissue cyst.	Rodents play an important role in the transfer of zoonotic parasite diseases. Control of rodent population in the city should be considered to decrease risk of transmission.	[30]

Abbreviations: bp, base pair; ELISA, enzyme-linked immunosorbent assay; F, forward; ICA, immunochromatographic assay; IFA, immunofluorescence assay; MAT, modified agglutination test; *M. musculus*, *Mus musculus*; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; R, reverse; *R. rattus*, *Rattus rattus*; *R. norvegicus*, *Rattus norvegicus*; *T. indica*, *Tatera indica*.

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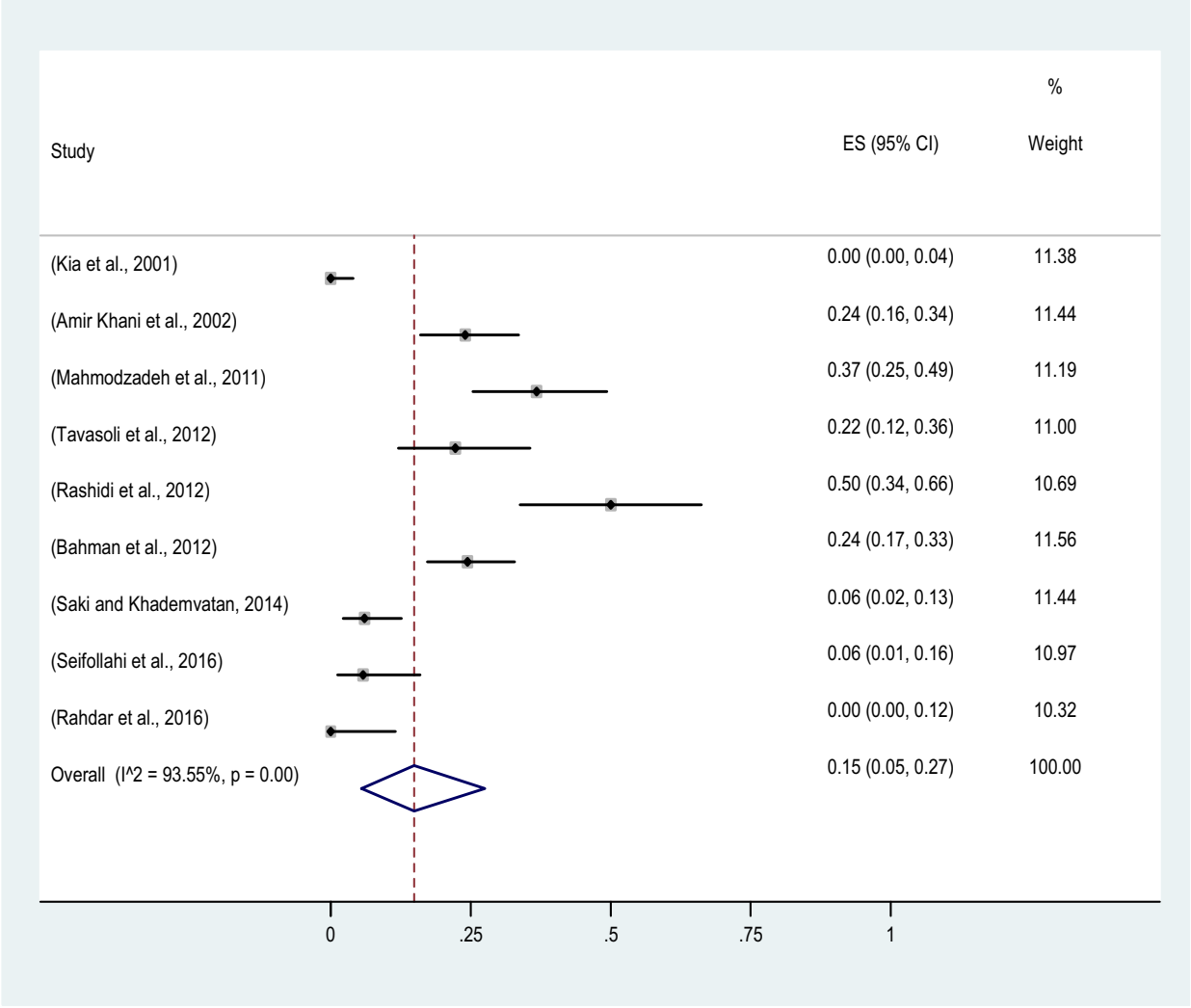

Fig. 2. Forest plot diagram of the current systematic review and meta-analysis based on overall prevalence.

selected based on title and reviewed carefully to assess the eligibility for inclusion. Afterwards; those papers were included in this systematic review that estimated the prevalence of *Toxoplasma* in rodents using at least one of the following laboratory diagnostic methods including: direct microscopy examination; serologic tests and PCR-based techniques. Finally; the required data were recorded using a data extraction sheet on the basis of title; province; first author; sample size; prevalence rate; kind of diagnostic methods (direct microscopy examination; molecular and serology); some details; main results or suggestions and reference. The PRISMA protocol (preferred reporting items for systematic reviews and meta-analysis) was followed to report our finding [28]. Meta-analysis procedure was performed as formerly described by us [8,11].

Results

A total of 291 citations were retrieved following the initial search of databases and finally, 9 of them had eligibility to be included in current systematic review and meta-analysis [29–37] (Fig. 1). The findings of included articles have been inserted in Table 1. Due to significant heterogeneity, the random-effects model was used (I^2 = 93.55%). Moreover, Egger's regression test showed no significant publication bias (P = 0.83).

A total number of 661 wild rodents were trapped during 1 January 2000–10 September 2016 and examined for the presence of *T. gondii*. Of these, 121 rodents were diagnosed positive and overall weighted prevalence of toxoplasmosis was estimated 15% (95% CI = 5–27). The forest plot diagram of present systematic review and meta-analysis is depicted as Fig. 2. For this purpose, three different laboratory diagnostic tests were employed including: direct microscopic examination, molecular test and serologic assays. Briefly, in subgroups, overall prevalence using direct microscopic examination (three studies—1/230), PCR-based techniques (four studies—41/246) and serological tests (5 studies—83/437) was obtained 0.1% (95% CI = 0.0–1.5), 18% (95% CI = 4–39) and 15% (95% CI = 3–33), respectively (Tables 1 and 2). Meta-regression analysis demonstrated that there is no significant relationship between overall prevalence with sample size (P = 0.52) and year of publication (P = 0.91).

Discussion

Although more than 100 years have passed from the discovery of *T. gondii*, from an African rodent, *Ctenodactylus gundi* [3]; this parasite still possess a relatively high prevalence in men and different animal hosts [1]. It is estimated over one-third of the world's population are infected with *Toxoplasma* and carry it [1,2,11,18]. In recent years, the prevalence of toxoplasmosis in var-

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Table 2
Subgroup analysis for comparison of prevalence based on diagnostic methods.

Method	No. of studies	Prevalence (95% CI)	I^2%	Heterogeneity test		Egger test	
				Q	P	t	P
PCR	4	18% (4–39)	92.22	38.55	<0.001	1.61	0.249
Serology	5	15% (3–33)	94.86	77.76	<0.001	−0.36	0.744
Direct microscopy examination	3	0.1% (0.0–1.5)	0.00	1.005	0.605	−0.36	0.778

ious human groups and animal species have been investigated comprehensively in Iran country as follows: general population 39% (95% CI = 33–46) [22], pregnant women 41% (95% CI = 36–45) [8], immunocompromised individuals 50% (95% CI = 44–56) [14], apparently healthy blood donors 33% (95% CI = 24–42) [11] and animals such as cats 34% (95% CI = 22–46) [4], sheep 31% (95% CI = 26–35) [20], goats 27% (95% CI = 14–42) [20] and cattle 18% (95% CI = 10–28) [21].

In present systematic review, we studied the prevalence of *T. gondii* in rodents of Iran. During the years (1 January 2000–10 September 2016), 121 out of 661 captured rodents, were identified positive for parasite and rate of prevalence was calculated 15% (95% CI = 5–27) based on random-effects model (Fig. 2). It is evident that domestic and peri-domestic rodents are the greatest and diversified order of mammals in the earth, and contributes in the ecological food chain and spread of parasites such as *Toxoplasma* to other animal hosts [24,25]. Overall, the importance of rodent species in the life cycle of *Toxoplasma* is misapprehend, depended on influence of ecological factors [24,38]. While, rodents are one of the reservoir hosts for *Toxoplasma*, mostly preyed by cats and dogs, hence can spread the parasite to other animal hosts, as well. Besides, rodents with maintenance *T. gondii* transmission cycle in surrounding regions, plays a main role in morbidity and incidence of human and livestock, especially in crowded regions [24]. *Rattus rattus*, *Rattus norvegicus* and *Mus musculus* can be found worldwide and have been often investigated in multiple countries [24–26,31,39].

In Pakistan with 52% (123/210—58.6% for rats and 33/90—36.7% for mice) [40], Philippines 55% (87/157) [41] and Serbia 27.5% (22/80) [42], prevalence rate of toxoplasmosis in rodents was higher than our study 15% (95% CI = 5–27—121 positive out of 661 tested); while lower prevalence was reported from Niger 1.96% (15 positive out of 765 tested) [43], Tanzania 2.17% (1/46) [44], France 4.08% (29/710) [45], Netherlands 4% (10/250) [46], Thailand 4.6% (21 positive out of 461 examined) [38] and Southern China 3.2% (7 positive out of 217 tested) [47]. The discrepancy between countries might be originated from differences in cultural habits, hygienic level, population distribution, abundance of intermediate and definitive hosts and respective distribution, nutrition habits of nations, etc. [24,39,48]. Consumption of infected rodents in some nations increased the rate of transmission from rats to men, as it has been documented dramatically higher frequency of infection in those people that eat or handle the rats [39,48,49]. Exposure to *Toxoplasma* in rodents is impressed by various environmental circumstances and depended on own ecological niche of each rodent species and their animal predators [24].

Based on present findings, only 1 out of 230 examined samples was diagnosed positive using microscopic detection 0.1% (95% CI = 0.0–1.5), however using PCR-based methods, 41 out of 246 samples 18% (95% CI = 4–39) was *T. gondii*-positive. As it was reported, direct microscopic observation is not a suitable and reliable method to detect *T. gondii* alone [30,31]. Also this method has low sensitivity and needs to expertise [31,42]. While PCR-based techniques with high sensitivity, specificity and rapidly features are more appropriate for diagnostic purposes in different tissues (brain, muscle, blood, etc.) [31–33]. The reason for observed discordance between molecular methods and microscopic detection, is differences in their technical nature [2,42]. In current study, serological tests showed acceptable results 15% (95% CI = 3–33–83 positive out of 437 tested), approximately similar to molecular methods (Table 2).

It should be noted, five major limitations confined our insight and knowledge about the prevalence of toxoplasmosis in captured rodents. (1) Only few number of prevalence reports were available from Iran; (2) lack of evaluate related risk factors in articles such as sex (m/f), site of captured rodents (urban/rural), wild or pet rodents, etc.; (3) lack of studies in major parts of country (only studies were found from 5 out of 31 provinces); (4) the retrieved papers were utilized a variety of laboratory methods such as microscopic detection, molecular and serological tests with different specificities, sensitivities or cut off levels; (5) prevalence data were often based on sampling from limited trapped rodents in limited regions. The epidemiological figures of toxoplasmosis in rodents, is influenced by above-mentioned limitations, and to minimize the fault, more rigorously studies should be designed in future.

In conclusion, to the best of our knowledge, this was the first systematic review in rodents of Iran, an overlooked hosts of *T. gondii*. The relative high exposure of *Toxoplasma* in rodents of Iran 15% (95% CI = 5–27), may hypothesize the presence of congenital transmission of parasite to sustain of infection within colonies of rodents, as it was experimentally confirmed [50]. Rodents acts as a key component in the life cycle of *T. gondii* and should not be ignored. However, the implication of wild and domestic rodents in the epidemiology of toxoplasmosis is not fully understood and therefore this matter needs more attention throughout the globe and, of course in Iran. It is highly proposed further investigations on those parts of Iran with high endemicity (particularly, northern and southern parts of country) for the aim of better understanding the role of different rodent species in the epidemiology pattern of toxoplasmosis.

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Competing interests

None declared.

Ethical approval

Cod: IR.UMSU.VEC.1395.455.

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