Cattle toxoplasmosis in Iran: a systematic review and meta–analysis

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ABSTRACT

Objective: To analyze and review the overall seroprevalence rate of Toxoplasma gondii (T. gondii) infection in cattle from Iran. Methods: In the current study, data collection (published and unpublished papers, abstracts of national scientific congresses and dissertations) using particular terms was carried out systematically on the following electronic databases like PubMed, Google Scholar, Ebsco, Science Direct, Scopus, Magiran, Irandoc, IranMedex and SID (Scientific Information Database). Results: A total of 22 studies since 1983 to 2012 reporting the seroprevalence of toxoplasmosis in cattle from different regions of Iran met our eligibility criteria. The pooled proportion of toxoplasmosis, using random effect model, among cattle in Iran from over the 30–year period was estimated 18.1% (95% CI: 9.9% to 28.2%) . Conclusions: This study firstly establishes a crude seroprevalence rate of Toxoplasma infection in cattle which can lead us to understand the condition of cattle toxoplasmosis, which have to take into accounted for an appropriate and effective prevention and controls. Secondly, it compares and discusses elaborately the role of risk factors including sex, age and breed in the epidemiology of the disease. Thus, it determines gaps and drawbacks in the prior studies which are greatly useful to design more accurate investigations in the future.

1. Introduction

Toxoplasma gondii (T. gondii) is a causative agent of toxoplasmosis which was first described by Nicolle and Manceaux in 1908 from North African rodent (Ctenodactylus gondii)[1]. This cosmopolitan parasite is considered as an obligatory intracellular protozoan infecting a wide variety of blood warmed vertebrates including human being and cattle as intermediate hosts. First case of Toxoplasma infection in cattle occurred in Ohio, U.S.A. in 1953[2]. Felids play a major role in epidemiology of this zoonotic disease as final hosts. Felids are the solely definitive hosts which excrete oocysts

in their feces. It was mentioned that newborn kittens are more dangerous compared to adult cats for transmission of this infectious disease[3,4].

Toxoplasmosis is an important matter not only in medical but also in veterinary field. It is estimated that approximately one– third of the human globe population is infected with T. gondii[5]. The overall seroprevalence rate of toxoplasmosis among the general population in Iran was 39.3% (95% CI=33.0%–45.7%)[6]. Toxoplasmosis causes significant economical losses and damages in animal husbandry. This parasite achieved veterinary importance when it was found to induce abortion storms in sheep in Australia, 1957 and also other economical damages due to stillbirth and neonatal mortality in sheep and goats in other parts of the world[7,8]. Toxoplasma is considered as a foodborne risk and in Iran, infection is observed in many domesticated animals including cattle, lambs and goats which are used as food material sources. Aside from

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consuming food or drink contaminated with oocysts of *T. gondii*, either eating undercooked or raw meat of ruminants are evidently being a prime source of infection for human and ingestion of under–cooked beef is considered as a risk factor for human toxoplasmosis[5].

Though numerous and various studies have been undertaken, the relative contribution of foodborne (meat) sources against oocyst transmission of *Toxoplasma* infection to human is still remained unknown. This point is worthwhile to mention that epidemiological investigations still are the most useful methods for evaluating the relative importance of different sources of *Toxoplasma* infection in humans. To the best of our knowledge, despite of a large number of epidemiological surveys on animal toxoplasmosis in Iran, there is not any comprehensive and documented systematic review and meta–analysis on seroprevalence of toxoplasmosis in cattle.

Therefore, the objective of the current systematic review and meta–analysis was to determine the weighed seroprevalence of *T. gondii* infection and describe the epidemiological transmission of infection in cattle of Iran.

2. Materials and methods

2.1. Database search

For the purpose of gathering information, a precise and comprehensive search was performed on all scientific publications (full texts and abstracts) from October to December in 2012 and all process was presented in Figure 1. Nine included databases were as following: five English databases (PubMed, Google Scholar, Ebsco, Science Direct and Scopus) and 4 Persian databases (Magiran, Irandoc, IranMedex and SID). In addition, dissertations and all abstract books of scientific congresses in Iran from 1983 to 2012 were evaluated carefully. In order to avoid missing any articles, whole references of papers were meticulously checked as well.

The search terms which were used alone or combined were "*T. gondii*", "toxoplasmosis", "*Toxoplasma* infection", "animal toxoplasmosis", "cattle", "buffalo", "epidemiology", "seroprevalence", "Iran", "meat producing animal" and "anti–*Toxoplasma* antibodies". Moreover, Language of data collection was limited to Persian and English.
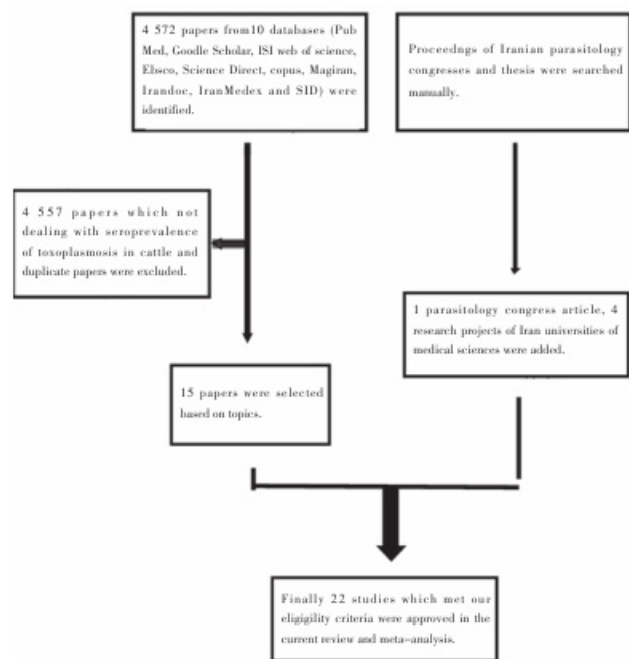

Figure 1. Flowchart describing the study design process.

2.2. Data collection

All cross–sectional studies that were carried out to estimate the prevalence of toxoplasmosis, diagnosed by serological methods, on cattle (alive, slaughtered, aborted or meat products) were included. Repetitive papers were excluded. Furthermore, the collected data for the current study were as follows: year of publication, first author, study areas, sample size, number of males and females, prevalence, age of samples, diagnostic test and time of conducting study. For this purpose a data extraction form was used.

2.3. Statistical methods

Both the crude and the weighted prevalence estimate and their 95% confidence interval for each included study were calculated. Forest plot was used to visualize the heterogeneity among studies. The heterogeneity was expected in advance and statistical methods, I^2 and Cochrane Q statistics (with significance of $P<0.1$) were used to quantify the variations. For the purpose of meta–analysis we assumed that the included studies are a random sample from a population of studies and a random effect model was employed. Proportions of individual studies and overall prevalence were presented by forest plot. The meta analysis was performed with the trial version of StatDirect statistical software (http://statdirects.com).

3. Results

From the nine databases, 22 studies met the eligibility criteria, included in this systematic review and meta-analysis as lined in Table 1. A total number of 6 869 cattle were examined for toxoplasmosis from 1983 to 2012 in different areas of Iran and 993 cases were diagnosed positive using different serological diagnostic tests as presented. During 30 years, seven different types of diagnostic tests were employed to evaluate *Toxoplasma* infection in cattle as following: modified agglutination test (MAT), enzyme-linked immunosorbent assay (ELISA), indirect haemagglutination test (IHA), direct agglutination test (DAT), Indirect Immunoflourescent assay (IFA), latex agglutination test (LAT) and Sabin & Feldman test (SFT). Comparison of sensitivity and specificity of all above mentioned diagnostic laboratory methods for toxoplasmosis was indicated in Figure 2. The most used diagnostic method for *Toxoplasma* infection in cattle in Iran was IFA test (8 studies) which was followed by MAT (5 studies), LAT (3 studies), DAT (2 studies), SFT (2 studies), IHA test (1 study) and ELISA (1 study). The pooled proportion of toxoplasmosis, using the random-effect model, among cattle in Iran from 1983 to 2012 was estimated 18.1% (95% *CI*: 9.9% to 28.2%) and forest plot diagram of current study was depicted in Figure 3. A wide variation in the prevalence estimates between different studies was observed (*Q* statistic= 1 982.9, *df*=19, *P*<0.0001) and I^2=99%. Seropositive rate of cattle toxoplasmosis in various regions of Iran was between 1.4% and 71.3% in Kerman and Tehran Province, respectively. During 3 decades, nearly half of included investigations (12 out of 22) were performed on cattle in Tehran (6 studies and *n*=539) and Khozestan Province (6 studies and *n*=1 896). Prevalence rate of toxoplasmosis in cattle in different parts of Iran was shown in Figure 4.

Figure 3. Forest plot diagram of current systematic review and meta-analysis.

Figure 4. Prevalence of toxoplasmosis in cattle in different provinces according to antibody seropositivity.
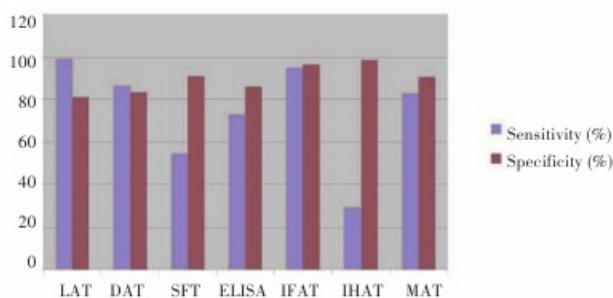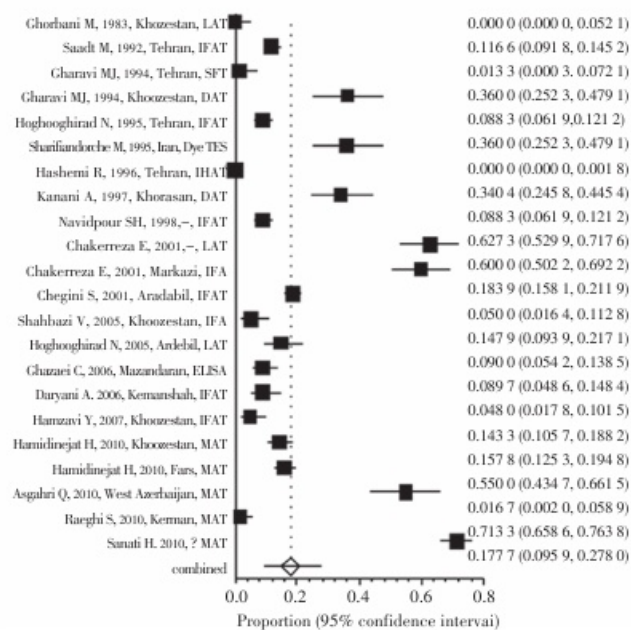

Figure 2. Comparison of different diagnostic methods based on their sensitivity and specificity.
LAT [48]; DAT [49]; SFT [50]; ELISA [50]; IFA [18]; MAT [50].

4. Discussion

This study was designed using 10 data bases, 22 records, 6 946 cattle and 980 seropositive cases. Our results indicated that the seroprevalence of toxoplasmosis in cattle of Iran during 30 years was 18.1% (9.5%–28.2%). Moreover, the highest and the lowest prevalence were recorded in Kerman, 71.3% and Tehran, 1.4%[9,10]. This point is worthwhile to mention that the worldwide seroprevalence of toxoplasmosis in cattle was estimated 9%[11]. The prevalence of *Toxoplasma* infection of cattle in neighbor countries were as following:

Table 1
Included publications of cattle toxoplasmosis for meta–analysis.

No.	Year of publication	First author	City	Province	Diagnostic method	Total individuals	Positive individuals	Seroprevalence %	Reference
1	1983	Ghorbani M	Iran*	Iran*	LAT	69	0	0.0	[33]
2	1992	Saadat M	Ahvaz	Khozestan	IFAT	592	69	16.2	[34]
3	1993	Hoghooghirad N	Ahvaz	Khozestan	LAT	142	21	14.8	[35]
4	1994	Gharavi MJ	Tehran	Tehran	SFT	75	1	1.4	[36]
5	1994	Gharavi MJ	Tehran	Tehran	DAT	75	27	36.0	[36]
6	1995	Sharifidorche M	Tehran	Tehran	SFT	75	27	36.0	[37]
7	1996	Hashemi R	Iran*	Iran*	IHAT	200 0	0	0.0	[38]
8	1997	Kanani A	Tehran	Tehran	DAT	94	32	34.0	[39]
9	1998	Navidpour SH	khozestan	khozestan	IFAT	385	34	8.8	[40]
10	2001	Chakerreza E	Tehran	Tehran	LAT	110	69	62.8	[41]
11	2001	Chakerreza E	Tehran	Tehran	IFAT	110	66	60.0	[41]
12	2001	Chegini S	Saveh	Markazi	IFAT	832	153	18.4	[42]
13	2003	Mohammad Zadeh M	Meshginshahr	Ardabil	IFAT	100	5	5.0	[21]
14	2006	Daryani A	Mazandaran	Mazandaran	IFAT	145	13	9.0	[43]
15	2006	Ghazaei C	Ardabil	Ardabil	ELISA	200	18	9.0	[22]
16	2007	Hamzavi Y	Kermanshah	Kermanshah	IFAT	125	6	4.8	[44]
17	2008	Nematollahi A	Tabriz	East Azarbaijan	IFAT	490	78	15.9	[45]
18	2010	Hamidinejat H	Ahvaz	Khozestan	MAT**	300	43	14.3	[46]
19	2010	Hamidinejat H	Ahvaz	Khozestan	MAT***	450	71	15.7	[47]
20	2010	Asgar Q	Fars	Fars	MAT	80	44	55.0	[20]
21	2011	Raeghi S	Urmia	West Azarbaijan	MAT	120	2	1.6	[9]
22	2012	Sanati H	Kerman	Kerman	MAT	300	214	71.3	[10]

*: In Iran , * * : cut off: 1/20 and * * * : cut off: 1/25.

In Turkey, a study using SFT method in Vezirkopru and Bafra areas showed that the prevalence were 66% and 39.5%, respectively[12]. Whereas in another similar investigation, 93.5% of samples in the Kars Province, Turkey were detected positive by ELISA method that was noticeably higher and two above mentioned surveys are not consistent with our findings[13]. In Kaboul, Afghanistan, consistent with our finding, 20.4% of buffaloes by IFAT were detected positive for IgG antibody to *T. gondii*[14]. Besides, Al–Ramahi in Mid–Euphrates region–Iraq detected antibody of *T. gondeii* in 29.2% of examined samples by LAT[15]. In all above mentioned surveys, the prevalence was higher than our results.

High prevalence of toxoplasmosis of cattle in some areas may be due to the following factors: humid and temperate climate; the absence of routine treatment against feline toxoplasmosis, considerable cat abundance and last but not least exposure to cats and their oocysts.

It was demonstrated that there is a meaningful relationship between climate and toxoplasmosis prevalence. It is usually more prevalent in warm, humid climates and lower altitudes compared to cold or dry districts. This fact is associated to longer viability of *T. gondii* oocyst in warm and humid surrounding[16]. In addition to regional climates, this difference may originate from sample size, different sensitivity and specificity of applied methods. Therefore, heterogeneity is seen in the forest plot diagram that it supports the reason of applying the random effect model.

In the past, different serological diagnostic tests were utilized in the majority of *Toxoplasma* surveys in cattle due to their reliable sensitivity. In Iran, nearly half of surveys have been done using IFA. This test was introduced

in 1992 and considered more reliable test compared to other serological diagnostic methods due to its significant sensitivity and specificity. This method is relatively simple assay for evaluating the infection of animals, also is particularly useful test for screening a large number of specimens and aforementioned facts may be explained why the majority of studies have used this method[17,18]. Besides, MAT test has been performed for toxoplasmosis diagnosis in cattle in recent years. Today, this form of agglutination test is broadly utilized for research purposes of *T. gondii* both in humans and all species of animals. Besides, this rapid and easy test just requires initial facilities[19]. Furthermore, both ELISA and IHA methods were used only in one study.

In some areas such as Kerman and Fars which are neighbors the prevalence was considerably high compared to other parts of Iran, 71.3% and 55%, respectively[10,20]. Furthermore, the prevalence in Urmia, Meshginshahr and Ardabil which are located in North West of country were relatively low 1.6%, 5% and 9%, respectively[9,21,22]. This low prevalence may attribute to this fact that all three mentioned areas are cold and mountainous regions which are not in favor of oocyst sporulation and survival.

The age of animals is a major factor for prevalence of toxoplasmosis. Since, young animals were less infected than older animals. Indeed, it is expected that with a raise in age of animal, exposure to *Toxoplasma* infection increases as well. In contrast, Navidpour and Hoghooghi–Rad and Saadat indicated that prevalence in cattle under one year old was higher compared to older than one year[34,40].

It is important to bear in mind that although *T. gondii* has been isolated frequently from mutton, investigation for finding cysts in cattle or buffalo tissues revealed rare cases while seroprevalence study demonstrated very high antibodies detection in cattle[23–25]. In contrast, based on some case–control studies, ingestion of under–cooked beef is considered as a risk factor for human toxoplasmosis despite above mentioned results[26,27]. Experimentally inoculated cattle surveys revealed a higher antibody titer in calves than cows. Additionally, Nematollahi and Moghddam pointed that calves are more susceptible than adults[45]. Some major clinical manifestations of orally affected calves comprise fever, weakness, poor weight gain, depression, diarrhea, anorexia, dyspnea and mild lymphadenopathy. Signs of calve with congenital infection include fever, cough, sneezing, dyspnoea, neurological signs, stillbirths and neonatal deaths. Usually the most serious toxoplasmosis symptoms in adult are dyspnoea, fever, and nervous

signs and lethargy[28]. An acceptable justification for the susceptibility and serious symptoms of calves is that the young animals have an immature immune system and this fact may put them more at risk of serious infection compared to adults.

Although the breed of examined samples plays a significant role in epidemiology of toxoplasmosis, this important factor was noted only by Navidpour and Hoghooghi–rad and Hamidinejat *et al* that water buffaloes (Bubalus bubalis) were examined for *Toxoplasma* infection[40,46].

Cow's milk is another primary source of nutrition for human. Although, acute symptoms of human *Toxoplasma* infection just has been related to using unpasteurized goat's milk, tachyzoites of *T. gondii* have been reported in the milk of cows[23,29–31]. Since, diagnosis of infected dairy cow with the disease is a matter of high importance.

There is a different susceptibility to *T. gondii* infection in cattle and sheep. Because in pregnant sheep either abortion or birth of weak lambs induced due to *T. gondii* infection whereas cattle infected by parasite showed no abortion or prenatal mortality[32]. Therefore, cattle are resistant to *Toxoplasma* infection owing to more effective immune response compared to sheep.

Livestock acquire *Toxoplasma* infection merely via ingestion of oocyst and when prevalence is considerably high. It shows widespread oocyst contamination of the environment because of faecal contamination of soil and groundwater either by domestic or feral cats[5]. And this fact put human more at danger of getting *Toxoplasma* infection and it can be considered as an alarm for human community because it elevates the chance of getting infection. Understanding prevalence rate of animal toxoplasmosis will help us to estimate the rate of human toxoplasmosis and it can be a good indicator of environment and final host contamination. This point is extremely important to mention that it is not easy to consider prevention and control program without enough information about prevalence of toxoplasmosis in animal owing to being as a major source of transmission to human. The importance of expanding our knowledge about condition of cattle toxoplasmosis originates from their vital role owing to their products (meat and milk) in public health and the prominent role of definitive host in disseminating and contaminating the environment by oocyst.

In conclusion, this study accentuates some valuable and interesting points: firstly, the seroprevalence rate of toxoplasmosis in cattle in Iran is relatively high (18.1%) and this considerable rate is associated with a high

contamination of environment by final host oocysts. Hence, continuous serological researches and preventive measures should be taken into consideration owing to the significance of the disease. Secondly, our data are limited just to nine out of thirty one provinces and there is not enough data for cattle toxoplasmosis in majority of provinces and further investigations are highly suggested in order to fill this remarkable gap and complete other parts of this puzzle. Thirdly, albeit many efforts have been made to determine the seroprevalence of toxoplasmosis in cattle in different parts of Iran, some gaps in prior studies was obviously clear. Not enough attention was paid to the role of major factors including sex, age and breed of examined cattle despite of their key role in epidemiology of the disease. Hence, considering all above mentioned parameters are required in order to overcome these shortcomings in the future.

Conflict of interest statement

We declare that we have no conflict of interest.

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