



Original Research Article

Detection *Toxoplasma gondii* by Latex and ELISA Test in Infertile and Fertile Men in Kalar City, Kurdistan Region, Iraq

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ABSTRACT

Keywords

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The current study was performed in Kalar city, Kurdistan Region, Iraq, through the period from November 2013 to June 2014. This study was done on a total of 260 persons attending to the laboratory of the General Hospital of Kalar city. Latex agglutination test (LAT) and enzyme linked immunosorbent assay (ELISA) were used to detect the prevalence of anti-*Toxoplasma* IgM and IgG antibodies, to estimate the relation between toxoplasmosis and age, occupation, is having contact with animals, residence, and number of children. Results indicated that there is a relationship between the above epidemiological factors and toxoplasmosis. Our results found an association between toxoplasmosis and secondary infertility. The prevalence of *T. gondii* in primary infertile, secondary infertile and fertile males was 45.16%, 53.33%, and 47.37% by using ELISA tests, respectively, while the distribution of non-infected primary infertility, secondary infertility and fertile males were 54.83%, 46.66% and 52.66% by ELISA tests, respectively.

Introduction

Toxoplasmosis, caused by the protozoan parasite *T. gondii*, is one of the most common parasitic infections of human and other warm-blooded animals. It has been found world-wide distribution (Hill and Dubey, 2002). Felines act as definitive hosts, while all non-feline vertebrates, including humans serve as intermediate hosts of *T. gondii* with disseminated tissue infections (Sudan *et al.*, 2013).

The main three infective stages of *T. gondii* are tachyzoite, rapidly dividing invasive, a slowly dividing bradyzoite in tissue cysts,

and an environmental stage, the sporozoite, protected inside an oocyst (Robert-Gangneux, 2013). Herbivores are infected by ingestion of food and water contaminated by oocysts, and carnivores by eating tissue cysts located in the meat of infected animals. Omnivores, including humans, are infected by both routes – by oocysts via unclean vegetables or fruits, contaminated water or hands, and by tissue cysts via improperly processed or raw meat (Bobic' *et al.*, 2012).

During the cycle of asexual multiplication, tachyzoites may disseminate absolutely to

any organ within the intermediate host, mostly to muscles, brain, placenta, udder and gonads (Gilot-Fromont *et al.*, 2012). Tachyzoites of *T. gondii* have been observed in body fluids, such as saliva, sputum, urine, tears, semen and milks of intermediate hosts, including sheep, goats and cows (Tenter *et al.*, 2000).

Male fertility can be reduced as a result of: congenital or acquired urogenital abnormalities, urogenital tract infections, increased scrotal temperature, endocrine disturbances, genetic abnormalities, immunological factors (Dohle *et al.*, 2010).

Infectious agents are able to interfere with the reproductive function of male and female, also infections of male genitourinary tract account for about 15% of the case of male infertility (Pellati *et al.*, 2008). There are many microorganisms such as; bacteria, fungi, viruses and parasites seem to be participating in male reproductive impairment (Pellati *et al.*, 2008).

T. gondii is a parasite that can infect male genital tract (Martinez-Garcia *et al.*, 1996). Toxoplasmosis is an important cause of bad obstetric history (BOH) leading to abortions and established its role as an agent of infertility (Malik *et al.*, 2014). The chronic toxoplasmosis showed an effect on men reproductive parameters (Eslamirad *et al.*, 2013).

Materials and Methods

Blood collection

Five ml of venous blood was taken from the males and two ml from the females in a 5 ml disposable syringe. After that blood transferred to 10 ml disposable sterile test tubes and put in incubator or room temperature for one hour at 37 °C for

clotting. The blood was then centrifuged at 3000 rpm for 10 minutes to separate the serum. The serum for males were transferred and divided into six disposable tubes, and those for females transferred and divided into two tubes. All isolated sera were tested serologically directly by latex for detection of *T. gondii* specific antibodies. After that, all positive and non-positive samples were kept in -45 °C until used to detect anti-Toxo IgG and IgM by Elisa tests.

Serological tests to detection of *T. gondii* infection

The sera of all cases were examined for the Toxo-Latex determination of antibodies anti-*Toxoplasma*. LAT kits were used; this kit was from linear chemicals Spectrum (Germany) and the presence of specific IgM and IgG anti-*Toxoplasma* antibodies via ELISA kits (Biocheck, USA) according to the manufacturer's Instructions.

Results and Discussion

The prevalence of infection with *T. gondii* in males according to age: results showed the highest seropositive percentage (80%) at age group of 46–50, 36–40 years and the lowest percentage was 50% at 20–25 years group by Latex test, while 62.5%, 53.84% at age group of >50, 41–45 years, respectively and there was not any infection at age group 20-25 years by ELISA tests, as shown in table 1.

The prevalence of infection with *T. gondii* in males according to occupation: results revealed the highest percentage were 85.71%, 81.81%, in mechanical, farmer group whereas the lowest was 50% in driver, office worker group by Latex test. While the highest percentage were 71.42%, 63.63% and the lowest was 33.33% in mechanical,

farmer group and assistant medicine group by ELISA IgG, IgM, respectively (Table 2).

The prevalence of infection with *T. gondii* in males according to region: result revealed higher percentage of *T. gondii* in males resides rural than urban habitat, the prevalence were 79.31%, 68.31% by Latex test and 58.62%, 44.55% by ELISA, respectively, as shown in table 3.

The prevalence of infection with *T. gondii* in males according to contact with animals: Results showed the higher prevalence of toxoplasmosis in males having contacted with animals than non-having contacted with animals, the percentage were 77.41%, 68.68% by Latex test and 61.29%, 43.43% by ELISA tests, respectively. In addition the prevalence of infection in infected males having contacted with cattle were 100%, dog 72.72%, birds 66.66%, sheep 62.5%, and cat 57.89% by ELISA, as shown in table 4.

The prevalence of infection with *T. gondii* in males according to the number of children in the family: The study showed highest prevalence of *T. gondii* 66% in infected infertile males with multiple abortions group, and was 75% in group had ≥ 7 children in infected fertile males, while the highest rate of seronegative in non-infected males in the same group was in non-infected infertile and in non-infected fertile males 33.33% and 38.46%, respectively, as shown in table 5.

The prevalence of infection with *T. gondii* in infertile and fertile males: The seroprevalence of *T. gondii* in primary infertile, secondary infertile and fertile males were 70.96%, 76.66%, and 65.78% by using Latex test; while, they were 45.16%, 53.33%, and 47.37% by using ELISA tests, respectively, as shown in table 6.

Results showed a significant difference between the Latex test and ELISA tests and the prevalence of *T. gondii* was associated with the secondary infertility in the infertile males.

According to age results revealed that there was a positive relation between the age group and the percentage of having toxoplasmosis infection. Results were in agreement with Abdul-Aziz (2014) who revealed an increase in the prevalence of toxoplasmosis infection at age group 43-60 years toxoplasmosis in hemodialysis patients in Baghdad. The highest rate of *Toxoplasma* seropositivity in Erbil-Iraqi Kurdistan, among women was at age group 47-57 years old (Hamad and Kadir, 2013).

The current results may be explained by probability exposure to *T. gondii* with increase the age, may be due to the fact that most humans acquire infection as a result of longer exposure time (Al-Nahari and Al-Tammimi, 2010).

In contrast, the study was in disagreement with Siddiqui *et al.* (2014) who observed that the highest prevalence of infection in pregnant women, India, was at age group 21–30 years. In Iraq, Erbil city, the toxoplasmosis was the highest in women aged between 26 and 30 years (Khoshnaw, 2011). The reason may be those age groups are most active and the probability of having contact with one of the several routes of infection as they ages (Tawfic, 2013).

On other hand previously studies assessed by Muluye *et al.* (2013) among people living in Northwest Ethiopia; Ghana; Al-Shua'aibi (2012) in Iraq; Chiang *et al.* (2014) in Taiwan revealed that there were no significant difference between age and infection with *T. gondii*.

However, various serological tests may produce different results because of the inherent sensitivity differences between the serological tests, the use of different kits from different companies used by each researcher and their variable conditions, date of studies, and affected by different factors such as the type of exposure to the parasite, genetic background of the different parasites and the host, and the type of immune response elicited by the parasite probably, due to differences in the sample population and the study region (Opsteegh *et al.*, 2011; Djakovic, 2012; Ferreira *et al.*, 2014; Abbas, *et al.*, 2014) may be related to several other factors including cultural level, nutritional habits in each country and different geographical areas within one country, dissimilar nutritional and behavioral patterns of life and among ethnic groups living in the certain areas, climate condition, and possessing of cat (Tenter *et al.*, 2000; Abdi *et al.*, 2008; Al-Mayahi, 2011; Akhlaghi *et al.*, 2014).

According to occupation results were agreements with Jafari *et al.* (2012) in Iran, Tabriz city, who showed significant relation between infection and soil related jobs. Occupational exposure with the gardening soil has significant association with toxoplasmosis (Malarvizhi *et al.*, 2012; Senthamarai *et al.*, 2013). Kudakwashe and Yesuf (2014) indicated that 87.5% of farmers tested positive to toxoplasmosis while only 25% of merchants had the same results in Ethiopia.

In contrast, in Kirkuk city, Tawfiq (2013) showed no significant relation between toxoplasmosis and occupation, educational level. Prevalence of infection with *T. gondii* was not significantly related to maternal job in a Sicilian population, Italy (Puccio *et al.*, 2014). There were no significant differences between toxoplasmosis and the contact with soil, consumption of raw or undercooked

meat, raw vegetables or salad (Doudou *et al.*, 2014).

Having a garden or working in the yard increases the chances of oocyst transmission by soil contact (Jones *et al.*, 2006). The risk for *T. gondii* infection increased with age and was higher among foreign-born persons, have low educational level, lived in crowded conditions, and those who worked in soil-related occupations (Jones *et al.*, 2001).

Human infected by toxoplasmosis when oocyst-contaminated dust particles were inhaled or ingested (Dubey *et al.*, 2009).

Moreover, the inadequate hygiene, feeding habits and suitable climatic factors for sporulation and survival of oocysts in the environment might have additionally contributed for the high seroprevalence. The relative importance of the risk factors varies between countries due to differences in cultural patterns and climatic factors affecting oocyst survival. Study area, study population, sample size, age, sensitivities of serological techniques employed, cat densities in the areas and access of cat to contaminate feed and water with oocysts and geographical variability may account for some of the differences in the reported seroprevalence (Gebremedhin *et al.*, 2013).

According to Region results were in agreement with Cai-xia *et al.* (2009) who revealed that rates of toxoplasmosis among men reside rural was 6.67% and then urban 2.35% in Shaanxi province. Among pregnant women in Amol, Northern Iran, the toxoplasmosis in urban areas was less than rural districts (Panah *et al.*, 2013). The *Toxoplasma* infection among pregnant women in Northeast Iran living in urban and rural areas was 29.1%, 47.5%, respectively (Babaie *et al.*, 2013).

On other hand, no significant relationship was seen between toxoplasmosis and living region (Bayani *et al.*, 2013; Sharbatkhori *et al.*, 2014; Akhlaghi *et al.*, 2014). This explained by the fact that transferring between rural and urban region will remove the difference between them.

In contrast, the current result was in disagreement with Mwabe *et al.* (2013); Sucilathangam *et al.* (2013); Kudakwashe and Yesuf, (2014) who showed higher infection rate of *T. gondii*- among pregnant women from the urban than those from rural communities. Risk for *T. gondii* infection increased with persons who lived in crowded conditions, and those who worked in soil-related occupations (Jones *et al.*, 2001).

Overall, the rural environment, and in particular farms buildings and their surroundings which shelter cats and intermediate host, agricultural activity are a major source of infection, including for other areas. In particular, rural areas and farms are the gate for *T. gondii* to circulate between the wild and domestic environments, thus their spatial distribution, management and level of biosecurity are determinant in the possibility for *T. gondii* to mix domestic and sylvatic cycles (Gilot-Fromont *et al.*, 2012). The prevalence associated to the residence in rural areas, low educational level, low family income, drinking water which does not originate from the public water supply system and the habit of handling soil or sand (Dias *et al.*, 2011). The pavement can reduce surviving period of oocysts in the urban area (Al-Saadii, 2013). Presence of large mechanical vectors, such as sow bugs, earthworms, and houseflies have been shown to contain oocysts, and cockroaches and snails are additional factors to prevalence of *T. gondii* in rural region (Dubey *et al.*, 2009).

According to contact with animals results of the current study were in agreements with the results of Ali (2008) who showed more cases of infected women among those who bred animals in their living places. *T. gondii* infection in Czech Republic military personnel was associated significantly with eating or tasting raw meat, owning a cat, owning rabbits, and owning a dog (Kolbecova *et al.*, 2007). The seropositivity of *T. gondii* was significantly associated with having contact with cat (Chiang *et al.*, 2014).

Sheep breeding and poor living were found to be statistically significant independent risk factors (Minbaeva *et al.*, 2013). The importance of knowing of the epidemiological situation of *T. gondii* infection in animals comes from the role of food milk, meat obtained from animals' intermediate hosts in public health and the role of cat as definitive host in contaminating the environment (Iovu *et al.*, 2009).

In aborted women in Kut city, Iraq, having contact with domestic animals cats, poultry, sheep, and goats showed high seropositive of *T. gondii* (AL-Mayahi, 2011). In Iran, Fars province the highest rate of infection 55% was found in the cattle followed by dogs 51.5%, horses 40%, sheep 29.5%, and goats 18.8%. This indicates that farm animals may play a major role in transmitting the infection to human through consumption of undercooked meats (Asgari *et al.*, 2013). *Toxoplasma* infection may be related to ethnicity and livestock species in Hongsipu district of Ningxia (Ju *et al.*, 2012).

There was high rate of infection with *T. gondii* in dogs (Wu *et al.*, 2011; Li *et al.*, 2012; Ferreira *et al.*, 2014). Dogs that commonly roll in cat feces can act as mechanical vectors in dissemination of

oocysts (Iovu *et al.*, 2009). The high prevalence of *T. gondii* infection in dogs in Tehran, Iran demonstrates high environmental contamination (Hosseininejad *et al.*, 2011).

In contrast, it was in disagreement with other studies Jafari *et al.* (2012); Senthamarai *et al.* (2013) and Doudou *et al.* (2014) they showed that there were no relations to contact with cats, breeding animals. Keeping cats out home, that reduces contact with cat feces, lead to prevent infection by *T. gondii* (Babaie *et al.*, 2013).

Since cows are one of the most important meat sources in Kerman Province, South East Iran, there is a high risk of contamination through meat from this host due to their susceptibility to infection (Sanati *et al.*, 2012).

The high rate of infects slaughtered sheep, suggesting them as a major risk factor of *T. gondii* via meat consumption (Boughattas *et al.*, 2014). Sheep could be an important source of *T. gondii* infection in humans (Cence-Guga *et al.*, 2013). Although the prevalence of *T. gondii* was low in cattle in the Netherlands, consumption of beef constitutes an important source of infection (Opsteegh *et al.*, 2011). Zhou *et al.* (2012) showed that *T. gondii* infection is prevalent in dairy cattle in southern China; which represents a potential source for human infection. Domestic birds in BeniSuef, Egypt are potential risk source of *T. gondii* infections in humans and animals (Aboelhadid *et al.*, 2013).

In organic livestock production systems, farm management factors play an important role in the farm prevalence of *T. gondii* (Meerburg *et al.*, 2006). The storage of animal feed may increase the presence of

rats that make the cats to spending length time looking for rats in the feed storage locations, and then cats will defecating on the feed contaminating it with oocysts (Gharekhani, 2013). A cat's natural instinct to bury or hide its feces provides millions of infective oocysts into the environment (Dubey *et al.*, 2009).

Cats are capable of roaming in various areas, including food storage areas and stalls. Oocyst contaminated pastures; fodder and drinking water are regarded as potential sources of postnatal infection in animals which has direct contact with farmer. Different serological tests, study design, climatic variations and frequency of felines and rodents in the farms are the main causes of these varied results (Heidari *et al.*, 2013).

According to the number of children in the family

The current study showed an association between the infected infertile males and multiple abortions which is explained by the occurrence of reactivation in the latent toxoplasmosis either in females or in males that may be transferred directly from the male to the females either orally or by semen rout, or the infection occurs via another routs of *T. gondii* transmission. Also the result of infected fertile in group ≥ 7 children illustrated the probability of chronic infection which remain for life span of the hosts or new acquired infection or reflected the small number of the samples participated in this group.

The result was close to Abdel-Hafez *et al.* (1986); Zargar *et al.* (1998) who suggested a possible causal relation between toxoplasmosis and repeated abortions. The high prevalence was detected in 19–24 years with a history of habitual abortions (Pavlinova *et al.*, 2011). The higher rate of

seropositive *T. gondii* antibodies was determined in serum of women with more than three abortions (Hamad and Kadir, 2013).

Pregnant women with three or more children had a higher seroprevalence of *T. gondii* infection (Ramsewak *et al.*, 2008). In China pregnant women toxoplasmosis had adverse outcomes including miscarriage, stillbirth, premature birth, and malformations (Li *et al.*, 2014).

In contrast other studies showed no significant relation between toxoplasmosis with children numbers of pregnancy (Tawfiq, 2013). Among pregnant women in Aydin province in Turkey, no relation was noted between toxoplasmosis and the number of abortion (Ertug *et al.*, 2005). Tachyzoites and DNA of *Toxoplasma* parasite were determined in sub mucosa and muscles of the uterus and in the villis of placenta, but did not indicate in histological sections of fetus (Bayat *et al.*, 2013).

The reasons may be due to the fact that humans are repeatedly exposed to the parasite and thus prevalence in the population increases with age (Pavlinova *et al.*, 2011), and in married females may be related to soil exposure since they are more involved with child rearing (Hamad and Kadir, 2013). There was correlation between toxoplasmosis and human infertility; *T. gondii* DNA was detected in genital tract of human, thus sex intercourse may be a rout of *T. gondii* infection between couples (Xiangcai *et al.*, 2000).

The prevalence of infection with *T. gondii* in infertile and fertile male

Latex test was accomplished to detect the type of *T. gondii* antibodies IgG, IgM by using ELISA technique because Latex test is a low sensitive test for detection of

toxoplasmosis, while ELISA is a highly sensitive and specific test (Al-Saadii, 2013). The IgM antibodies appear early before the IgG antibodies and disappear faster than IgG antibodies after recovery (Abbas *et al.*, 2014).

The Latex test was used to detect toxoplasmosis in serum because it is relatively simple, cheap and specific but less sensitive than other serological. Hence, ELISA IgG, IgM tests were used to more reliable results (Abdul-Aziz, 2014).

The Latex test result agreed with the previous study which is done by Hamza *et al.* (2011) in Karbala, Iraq and AL-Maamuri (2014) in Baghdad were recorded 67.7%, 75%, and 71.5%, respectively.

In relation, the current study was lower than those recorded by Abbas *et al.* (2014) who showed that the percentage of having the disease was 100% by Latex test and 59% were positive by ELISA (IgG, IgM) tests. The prevalence was 82% by Latex test, while the results of ELISA test by using anti-ToxoIgG revealed 60% (Dawood *et al.*, 2010).

In contrast, the current results were higher than a study done by Al-Saadii (2013) on male blood donors who recorded 34% in Baghdad by using Latex test. In china, in healthy population, the prevalence was 10.5% in men by using ELISA IgG test (Xiao *et al.*, 2010). It is shown that the rate of positivity by Latex 36.6% was significantly higher than ELISA 16.9% among pregnant women in Kirkuk city (Othman, 2004).

Toxoplasma infections was higher in primary infertility were 53.19%, 46.43% and 14.29% than in secondary infertility were 51.28%, 32% and 4% by using Latex and ELISA test IgG and IgM, respectively, while

in 2ME, the positivity rate in secondary infertility was slightly higher than those with primary type of infertility 15.79% and 14.29%, respectively (Hamad and Kadir,

2013). The reasons of variation may be related to the sample size or strain of the parasite (Hamad and Kadir, 2013).

Table.1 Prevalence of infection with *T. gondii* according to age

Age (year)	Total number of examined cases	Latex test		ELISA test	
		Number of positive cases	Prevalence of infection (%)	Number of positive cases	Prevalence of infection (%)
20 - 25	4	2	50.00	0	0.00
26 - 30	18	14	77.77	7	38.88
31 - 35	39	24	61.53	18	45.15
36 - 40	25	20	80.00	13	52.00
41 - 45	26	19	73.07	14	53.84
46 - 50	10	8	80.00	5	50.00
> 50	8	5	62.50	5	62.50
Total	130	92	70.76	62	47.69

Table.2 Prevalence of infection with *T. gondii* according to occupation

Occupation	Total number of examined cases	Latex test		ELISA test	
		Number of positive cases	Prevalence of infection (%)	Number of positive cases	Prevalence of infection (%)
Teacher	22	14	63.63	10	45.45
Assistant medical staff	9	5	55.55	3	33.33
Office worker	14	7	50.00	5	35.71
Police	22	18	81.81	10	45.45
Driver	8	4	50.00	3	37.50
Mechanic	7	6	85.71	5	71.42
Farmer	11	9	81.81	7	63.63
Building worker	37	29	78.37	19	51.35
Total	130	92	70.76	62	47.69

Table.3 Prevalence of infection with *T. gondii* according to residence

Region	Total number of examined cases	Latex test		ELISA test	
		Number of Positive cases	Prevalence of infection (%)	Number of positive cases	Prevalence of infection (%)
Urban	101	69	68.31	45	44.55
Rural	29	23	79.31	17	58.62
Total	130	92	70.76	62	47.69

Table.4 Prevalence of infection with *T. gondii* according to having animals

Animals	Total number of examined cases	Latex test		ELISA test	
		Number of positive cases	Prevalence of infection (%)	Number of positive cases	Prevalence of infection (%)
Cat	19	14	73.68	11	57.89
Dog	11	9	81.81	8	72.72
Cow	4	4	100.00	4	100.00
Sheep	16	13	81.25	10	62.50
Birds	9	7	77.77	6	66.66
Total number of contact with animals	31	24	77.41	19	61.29
Non-contact with animals	99	68	68.68	43	43.43
Total	130	92	70.76	62	47.69

Table.5 Prevalence of infection with *T. gondii* and its relation with the number of children in the family

Number of children	Total number of examined cases	Infected male				Non-infected male			
		Infertile		Fertile		Infertile		Fertile	
		Number of cases	Prevalence of infection (%)	Number of cases	Prevalence of infection (%)	Number of cases	Prevalence of infection (%)	Number of cases	Prevalence of infection (%)
0	62	27	43.54	0	0.00	35	56.45	0	0.00
1 - 2	33	5	15.15	7	21.21	7	21.21	14	42.42
3 - 4	13	1	7.69	6	46.15	1	7.69	5	38.46
5 - 6	4	1	25.00	2	50.00	0	0.00	1	25.00
≥ 7	4	2	50.00	3	75.00	1	25.00	0	0.00
Multiple abortion	12	8	66.66	0	0.00	4	33.33	0	0.00
Total	130	44	33.84	18	13.84	48	36.92	20	15.38

Table.6 Prevalence of infection with *T. gondii* in infertile and fertile males

Group	Number of examined cases	Latex test		ELISA test		
		Number of positive cases	Prevalence of infection %	Number of positive cases	Prevalence of infection %	
Infertile males	Primary	62	44	70.96	28	45.16
	Secondary	30	23	76.66	16	53.33
Fertile males		38	25	65.78	18	47.37
Total		130	92	70.76	62	47.69

Table.7 Total prevalence of infection with *T. gondii* in infertile and fertile male compared with non-infected male

Groups		Total number of examined cases	Infected male		Non-infected male	
			Number of cases	Prevalence of infection (%)	Number of cases	Prevalence of infection (%)
Infertile male	Primary	62	28	45.16	34	54.83
	Secondary	30	16	53.33	14	46.66
Fertile male		38	18	47.37	20	52.63
Total		130	62	47.69	68	52.30

On other hand in Baghdad, Abdul-Aziz (2014) showed that there were no significant differences between seropositive toxoplasmosis infections in hemodialysis patients by Latex test that was 39.0% and 35.75% by ELISA- IgG. The seropositive patients in general hospitals in Daejeon, Korea rates were 6.6% by Latex agglutination test and 6.7% by ELISA (Shin *et al.*, 2009).

The differences in the distribution of *T. gondii* infections may attribute to undercooked meat exposure or to cat feces, soil, level of education, climate conditions, cultural habits of the people, age, occupation, region residence, having animals and animal fauna (Dubey, 2010). Laboratory assays used, sample size, also increased globalization of the society, population increase, and shifts in human demographics may effect on the prevalence of *T. gondii* infection (Hill and Dubey, 2014).

The study showed that infection of *T. gondii* in infected males was associated with secondary infertility, as shown in table 7.

In contrast in non-infected males the cases related to the primary infertility. This result may be related to the number of samples, occupation, age, having contact with animals, residence of infertile samples, immunity of patients and duration of infection with *T. gondii*.

Results were in agreement with Malik *et al.* (2014) who indicated that the prevalence of *T. gondii* infection was more associated with secondary 66.7% than primary infertility 33.3% by using ELISA IgM. The rate of toxoplasmosis in Baghdad in the secondary infertile male showed 70%, while primary infertile male revealed 30% (Abdullah *et al.*, 2015). The rate of *Toxoplasma* antibody by ELISA 17.1% was in the infertile patients which was higher than 9.0% of normal controls (Jia-Zhou *et al.*, 2004).

The mechanism of the infertility with *Toxoplasma* in seminal plasma comprised production of lower acid phosphatase, seminal plasma inhibition material and positive anti-sperm antibody (Jiao *et al.*, 2001; Dao *et al.*, 2002).

T. gondii have been described in the male genital tract causing testicular damage or secondary hypogonadism via hypothalamic-hypophyseal axis alterations (Martinez-Garcia *et al.*, 1996). There is association of male genital tract impairment with special feature of testicular toxoplasmosis (De Paepe *et al.*, 1990).

The main changes detected were a focal mononuclear interstitial inflammatory infiltrate in the prostate and seminal vesicles; diffuse testicular degeneration associated with calcification foci and a multifocal mononuclear interstitial inflammatory infiltrate; and a mononuclear

interstitial infiltrate and focal necrotic areas of the muscle fibers surrounding the seminal vesicles (Lopes *et al.*, 2011). Primary infertility was higher in younger men while secondary infertility was higher in old men because they exposed to factors which disrupt their fertility (Abu Al-Haija, 2011). Thus toxoplasmosis may be one of those factors which cause secondary infertility.

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