Research Paper Medical Science

Association of Ifn-Gamma Gene Polymorphism With Infection of *Toxoplasma gondii* in Ewes in Some Area of Babylon Province- Iraq

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ABSTRACT

Eighty four aborted ewes from Babylon city were examined for detection antibodies of *Toxoplasma gondii* by using the latex test and ELISA test. Identification of IFN-γ A/T alleles were performed using specific primers and the method was the conventional PCR. The ewes were classified into subgroups according to age and months in order to study association of these risk factors with rate of infection. The latex method showed that the IgG was positive in 11 ewes (13.1%) while IgM was positive in 0 ewes (0.0%). The ELISA showed that the IgG was found in 74 ewes (88.1%) while IgM was found in 10 ewes (11.9%). There was no significant difference association between rate of infection in aborted ewes and risk factors: age and season. Also the results showed no significant difference association between allelic polymorphism of INF- γ gene and the rate of *T. gondii* infection in sheep.

KEYWORDS *Toxoplasma gondii,* ewes, abortion, IFN-gamma

INTRODUCTION:

The occurrence of *T. gondii* infection in animals was so vast so as to involve greater than 350 species, the greater majority being wild animals (1). The source of this wide range of infected hosts was explained by the shedding of the parasite Oocyst by stray and domestic felids (2). The rate of infection by *T.gondii* among meat producing animals has been reduced during the last decades by the effect of sufficient hygiene methods, keeping the animals in confined sheds a way from birds, cats and rodents and the good and sterile quality of food (3).

Cell mediated immunity was critical in human and animal resistance to *T. gondii* infection (4). When an infected cell, by *T. gondii*, subjected to IFN-γ it will undergo changes that permit elimination of the parasite (5). The mechanism by which elimination of the parasite established was through an autophagy-dependent fashion, this occurs by induction of transcription of p47 GTPases by the IFN- γ binding to cell receptor, GTPases then cause disintegration of the parasite vacuole allowing the enclosure of the parasite inside autophagosomes with subsequent destruction of the parasite by lysosomes (6).

The aim of the present study was serodiagnosis of *Toxoplasma gondii* in Babylon province by Latex and ELISA and the effect of polymorphism gene to IFN- gamma in ewes susceptibility to infection by *Toxoplasma gondii*. ALso study was investigate the association between rate of *T. gondii* infection in ewes and IFN-γ gene polymorphism.

MATERIALS& METHODS:

The study was designed to be an observational cross sectional and to involve 84 aborted ewes during May 2015 and extended through October 2015 in Babylon province. Blood samples were taken from all ewes. Hence the sample size was reduced to 84 ewes. Ewes were later on classified according to IgG ELISA results into 74 positive patients and 10 negative control gr oup.

Collection of blood samples

Five ml of blood samples were taken from the ewes, each sample was divided into two part, 3ml were put in tube contains gel material which will help to separate the serum which used for Latex and ELISA for IgG and IgM antibodies and 2ml in the EDTA tube was used for the purpose of extracting DNA to study IFN-γ gene polymorphism.

Primers

Primer sets have been used in ARMS PCR analysis of IFN-γ +874T/A gene polymorphism. The ARMS PCR primers for sheep was designed in this study by using NCBI.dbSNP database (dbSNP code: rs423751875) and BatchPrimer3 design program. These primers were provided from Bioneer Company. Korea as following tables.

Table 1: Sheep ARMS PCR primers

Primer	Sequence		Amplicon
Internal Control	F	AGGCTGATTCAAATTCCGGTGAGAGGAT	869bp
	R	CGAAAAATCTGGAGGCCTTTGTCCCTTA	
ARMS PCR	F	GGTGATGGGGGCAGAGTATTGAAAACCTAA	A/535bp
	R	AACAGAGACAAGGGGCAATGGGACAA	T/389bp

3.4. STATISTICAL ANALYSIS:

Data were summarized, presented and analyzed using statistical package for social science (SPSS version 16) and Microsoft Office Excel 2007. For the determination of the significant difference among one way analysis ANOVA was used. A p-value $p \leq 0.05$ was considered statistically significan the difference were considered statistically.

RESULTS:

The diagnosis of *T. gondii* in ewes was determined by two serological methods, Latex and ELISA. The latex showed that the IgG was positive in 11 ewes (13.1%) while IgM was positive in 0 ewes (0.0%). IgG identified cases with chronic infection while IgM identified cases with acute infection. The ELISA showed that the IgG was found in 74 ewes (88.1%) while IgM was found in 10 ewes (11.9%), as shown in table (2).

Table (2): Infection rate of *T. gondii* in ewes determined by ELISA and latex

		Positive		Total	
Test	Immunoglobulin	N	%	N	%
Latex	IgG	11	13.1	84	100.0
	IgM	0	0.0	84	100.0

Elisa	IgG	74	88.1	84	100.0
	IgM	10	11.9	84	100.0

Association between percentage of *T. gondii* infection and age

The results showed the percentage of acute infection in ewes (1-3) years of age was 16.3% while in ewes >3 years was 5.7%. The percentage of chronic infection in ewes (1-3) years of age was 87.8% whereas in ewes >3 years of age was 88.6%. There was no significant difference(P≥0.05) association between age of the ewes and percentage of infection neither acute (IgM) nor chronic (IgG), as shown in table (3).

Table 3: Percentage of infection in ewes with *T. gondii* according to age group

Age interval	Total number	IgM		IgG	
		N	%	N	%
1 -3 years	49	8	16.3	43	87.8
> 3 years	35	2	5.7	31	88.6
Total	84	10	11.9	74	88.1
P-value		0.255		0.909	

(P≥0.05)

The percentage of acute infection with *T. gondii* in ewes according to month

The rate of acute infection in May was 1 out of 14 (7.1%) which was similar to that of June and July. The percentage of infection in August was similar to that of October and was 2 out of 14 (14.3%), whereas the percentage of infection in September was 3 out of 14 (21.4%). There was no significant impact of the month of the year on percentage of acute infection difference, as shown in table (4).

Table 4: Effect of month of the year on percentage of acute infection with *T. gondii* in ewes.

Months	Total number	Infected cases	%	P
May	14	1	7.1	---
June	14	1	7.1	---
July	14	1	7.1	---
August	14	2	14.3	1.000
September	14	3	21.4	0.589
October	14	2	14.3	1.000
Total	84	10	11.9	---

(P≥0.05)

The association between γ-interferon gene polymorphism and percentage of *T. gondii* infection

There was no significant difference association between genotype AA and percentage of chronic infection (IgG seropositivity), P=0.376. The percentage of IgG seropositivty was 80% in AA γ-interferon genotype and 90.6% in other genotypes (TT and AT) collectively, as shown in table (5).

Table 5: The association between γ-interferon gene polymorphism and percentage of *T. gondii* chronic infection regarding AA genotype.

	IgG								
	Positive		Negative		Total				
Genotype	N	%	N	%	N	%	X2	DF	P
AA	16	80.0	4	20.0	20	100.0			
TT/AT	58	90.6	6	9.4	64	100.0	0.784	1	0.376
Total	74	88.1	10	11.9	84	100.0			

(P≥0.05)

The percentage of IgG seropositivty was 91.3% in AA γ-interferon genotype and 86.9% in other genotypes (AA and TT) collectively. There was no significant difference association between genotype AT and percentage of chronic infection (IgG seropositivity), P=0.857, as shown in table (6).

Table 6: The association between γ-interferon gene polymorphism and percentage of *T. gondii* chronic infection regarding AT genotype.

	IgG								
	Positive		Negative		Total				
Genotype	N	%	N	%	N	%	X^2	DF	P
AT	21	91.3	2	8.7	23	100.0			
AA/TT	53	86.9	8	13.1	61	100.0	0.032	1	0.857
Total	74	88.1	10	11.9	84	100.0			

(P≥0.05)

There was no significant difference association between genotype TT and percentage of chronic infection (IgG seropositivity), P=0.797. The percentage of IgG seropositivty was 90.2% in AA γ-interferon genotype and 86.0% in other genotypes (AA and AT) collectively, as shown in table (7).

Table 7: The association between γ-interferon gene polymorphism and percentage of *T. gondii* chronic infection regarding TT genotype

	IgG								
	Positive		Negative		Total				
Genotype	N	%	N	%	N	%	X^2	DF	P
TT	37	90.2	4	9.8	41	100.0			
AA/AT	37	86.0	6	14.0	43	100.0	0.066	1	0.797
Total	74	88.1	10	11.9	84	100.0			

There was no significant difference association between genotype AA and percentage of acute infection (IgM seropositivity), P=0.925. The percentage of IgM seropositivty was 15.0% in AA γ-interferon genotype and 10.9 % in other genotypes (TT and AT) collectively, as shown in table (8).

Table 8: The association between γ-interferon gene polymorphism and percentage of *T. gondii* acute infection regarding AA genotype.

	IgM								
	Positive		Negative		Total				
Genotype	N	%	N	%	N	%	X^2	DF	P
AA	3	15.0	17	85.0	20	100.0			
TT/AT	7	10.9	57	89.1	64	100.0	0.009	1	0.925
Total	10	11.9	74	88.1	84	100.0			

(P≥0.05)

There was no significant difference association between genotype AT and percentage of acute infection (IgM seropositivity), P=0.857. The percentage of IgM seropositivty was 8.7% in AA γ-interferon genotype and 13.1% in other genotypes (AA and TT) collectively, as shown in table (9).

Table 9: The association between γ-interferon gene polymorphism and percentage of *T. gondii* acute infection regarding AT genotype.

	IgM								
	Positive		Negative		Total				
Genotype	N	%	N	%	N	%	X2	DF	P
AT	2	8.7	21	91.3	23	100.0			
AA/TT	8	13.1	53	86.9	61	100.0	0.032	1	0.857
Total	10	11.9	74	88.1	84	100.0			

(P≥0.05)

There was no significant difference association between genotype TT and percentage of acute infection (IgM seropositivity), P=1.000. The percentage of IgM seropositivty was 12.2% in AA γ-interferon genotype and 11.6% in other genotypes (AA and AT) collectively, as shown in table (10).

Table 10: The association between γ-interferon gene polymorphism and percentage of *T. gondii* acute infection regarding TT genotype

	IgM								
	Positive		Negative		Total				
Genotype	N	%	N	%	N	%	X2	DF	P
TT	5	12.2	36	87.8	41	100.0			
AA/AT	5	11.6	38	88.4	43	100.0	0.000	1	1.000
Total	10	11.9	74	88.1	84	100.0			

(P≥0.05)

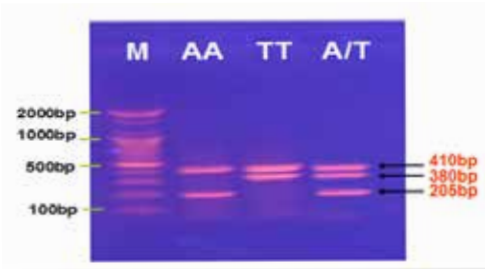

Figure 1: Agarose gel electrophoresis image that show the ARMS-PCR product analysis of IFN-g +874T/A gene polymorphism blood women samples. Where M: marker (2000-100bp), lane (1) AA homozygote at 410bp and 205bp product size, lane (2) TT homozygote at 410bp and 380bp product size, and lane (3) A/T heterozygote at 410bp, 380bp, and 205bp product size.

DISCUSSION:

The results of the present study showed that the sensitivity of ELISA was much better than Latex in detection seropositivity for IgG and IgM in sheep. These observations were coincided with (7) who stated that the sensitivity of ELISA was much better than Latex agglutination test in women. These our results may be indicated that ELISA can detect lower concentration of IgG and IgM. This explanation has been proposed by other authors (8). On other hands the current study reported that the rate of *T. gondii* IgG seropositivty in aborted ewes was 88.1%. in which was agreed with another Iraqi study that described the association between abortion in ewes and *Toxoplasma gondii* was 83.3% (9)

Globally, one of the most common causes of abortion in sheep is *T. gondii*, as it is the most frequent cause of sever fatal congenital abnormalities (10). In the United States of America the rate was estimated to be 17.5% (11); In Germany the frequency of abortion caused by *T. gondii* was 10.6% (12) these rates is much less than that reported in the present study. The rate of *T. gonddi* in ewes in relation to abortion in Iran was estimated to be around 32% (13). This is relatively higher than that of western countries, but it is less than that of the current study. The rate of seropositivty for anti *Toxoplasma* IgG in ewes with history of recurrent abortion was estimated to be around 85% and 61.4% in Egypt (14,15). These rates were considerably near the rate of our study.

These data were exhibited that there was a great economic loss in which related to the high abortion rate caused by *T. gondii* in ewes. The most important predisposing factor for this high rate of infection was the widespread contamination of water and food sources of Domestic animals, especially ewes. This contamination is caused by the extreme spread of infected cats, the major source of *Toxoplasma* Oocyts. Each acutely infected cat can pass millions of *Toxoplasma* Oocyst in their feces, causing widespread contamination of the environment. On the other hand the lack of appropriate measures to fight the source of infection by health authorities and veterinary institutes is a major participant in this high contamination (16).

The results showed that the age of the animal has nothing to do with the seroprevalence of *T. gondii*. This founding was disagreed with other to the worker (17). This may be due to less sample size and probably the high rate of infection at early age might be an acceptable explanation, nevertheless it should be pointed out that the present study showed a higher rate of IgG seroprevalence in older animals despite the absence of statistical significance. Seasonal variation was considered to have no effect on rate of *T. gondii* seroprevalence as stated. The founding of present study agrees with (18).

The explanation for that was related to several points. First of all IgG was present for long time in the individual animal and can be found at various months and seasons of the year. The source of acute infection, reflected by seropositivty for IgM, was mainly the contaminated food and water supplies that carry the infectious parasite Oocyst and because the period of study extended for a short period of time beside the strong resistance of *T. gondii* Oocyst to environmental factors, there was no significant seasonal difference in IgM seropositivty.

Our study showed no significant association between allelic polymorphism of INF- γ gene and the rate of *T. gondii* infection in sheep. The lack of association between allelic variations (A/T) of IFN- γ gene with the susceptibility to *T. gondii* infection may be explained as following: the homozygosity to A allele was associated with minimum level of IFN- γ production, nevertheless, this low level was sufficient to provide defense against *T. gondii* infection. Several studies have been conducted to discover the association between IFN -γ gene polymorphism and *T. gondii* ocular infection in human and revealed that patients who were homozygous for A allele were at higher susceptibility to ocular toxoplasmosis than patients with A/T or T/T genotype (19, 20).

Conclusion:

The intermediate level and high level of IFN- γ production by Heterozygous A/T and Homozygous T/T will provide high level of protection but will never make the rate of T. gondii infection less than persons who were Homozygous for A allele. That was to say there were other immune mechanisms that participate substantially in the defense against *T. gondii* infection, hence further studies were needed to disclose these mechanism.

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