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SEROLOGICAL AND MOLECULAR DETECTION OF *TOXOPLASMA GONDII* IN MEAT AND MINCED MEAT IN BASRA CITY

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ABSTRACT

The present study was conducted during the period between September 2017 and March 2018 to detect *Toxoplasma gondii* in meat. A total number of 387 blood, meat and minced meat were collected from cattle, sheep, goat and buffalo carcasses at slaughter house and butcher markets in Basra city. Molecular and serological methods were applied to detect the infection of *T. gondii* by using PCR and ELISA tests for all these samples. The results of ELISA showed that 25.32% of samples were positive, while 15.762% were positive for PCR test. Sheep showed the highest positive results for ELISA and PCR tests (33.036%, 22.321% respectively) flowed by goat (29.333%, 18.667%), cattle (25.439%,14.912%) and buffalo (11.628%, 4.651%) respectively. Positive ELISA and PCR results for minced meat (37.113%, 25.773% respectively) were higher than meat from both butcher markets (27.778%, 18.519%) and slaughterhouse (17.582%, 9.89%) respectively, while the results of ELISA and PCR conducted for blood analysis were 17.582% and 6.593% respectively. Higher toxoplasmosis ratio (32.195%, 21.951%) for ELISA and PCR respectively for samples collected from butcher markets in the present study.

INTRODUCTION

Toxoplasmosis is wide spread zoonotic disease caused by *Toxoplasma gondii*, the ubiquitous coccidian parasite of felines, man and many domestic warm-blooded animals (1). Infection of animals and humans with *Toxoplasma gondii* cause abortion, stillbirth and neonatal death (2,3).

The meat from infected animals is one of the most important potential source of human toxoplasmosis (4,5). Transmission of Toxoplasmosis to humans can occur by humans eat raw or inadequately cooked infected meat or eat uncooked foods (6).

Diagnosis is usually made by immunological testing and molecular techniques or by a combination of these techniques (7). Serological- tests such as ELISA that include indirect ELISA test which can define as a simple, rapid and accurate method for demonstration of IgM or IgG (8,9).

More specific, sensitive and reproducible detection of toxoplasmosis is improve the molecular diagnosis of the protozoan infection by polymerase chain reaction that used primers targeting B1 gene (10,11).

The Present study was aimed to evaluate two methods, ELISA and PCR to detect the overall prevalence of T. *gondii* infection in animals (sheep, goat, cattle and buffalo) from slaughterhouse and butcher markets.

MATERIALS AND METHODS

Sample collection

A total number of 387 blood, meat and minced meat samples were collected between September 2017 and March 2018 from cattle, sheep, goat and buffalo carcasses at slaughterhouse in Basra city (blood samples were collected before slaughtering and meat samples were collected after slaughtering from the same animal) and butcher markets in Basra city as describe in table 1.

Sera were obtained from blood samples by centrifugation and frozen immediately at -20˚C. 387 samples were tested molecularly to detect *T. gondii* tissue cyst (12).

Meat samples were minced thoroughly in sterile mincer. Meat and minced meat samples were allocated into sterile containers (20 ml) and frozen for 60 days at -

20°C. The frozen samples were thawed overnight at room temperature, and meat juice samples collected from the plastic bags. Meat juice and serum samples were submitted for serological analysis immediately after thawing (13).

Table (1) Blood, meat and minced meat samples collection

Sample	Cattle		Sheep		Goat		Buffalo		Total
	Slaughter house	Butcher markets	Slaughter house	Butcher markets	Slaughter house	Butcher markets	Slaughter house	Butcher markets	
Blood	28	0	25	0	18	0	20	0	91
Meat	28	31	25	33	18	22	20	22	199
Minced meat	0	27	0	29	0	17	0	24	97
Total	56	58	50	62	36	39	40	46	387

Detection of anti-*T. gondii* specific IgG

Detection of anti-*T. gondii* IgG antibody in meat, minced meat and sera samples were performed by using ID screen® toxoplasma indirect multi-species ELISA kit (ID. vet® France) according the leaflet of manufacture (14).

Molecular Detection of *T. gondii*

DNA extraction

The extraction of the DNA was done by using extraction kit (g SYNC tm DNA Extraction kit Quick protocol manufactured by Geneaid, UK) according to manufacture protocol.

PCR

The genomic DNA was subjected to PCR for amplifying B1 gene to detected *Toxoplasma gondii*. The primers sequence were listed in Table (2) and final product was 133 bp (15).

Table (2) The sequences of the primers used in the test

NO.	Target	Primer sequence (5'⟶3')	MT˚C	Pro. Size Base pair
1.	B1gene Forward	TTG CAT AGG TTG CAG TCA CT	56.5 C	133
2.	B1gene Reverse	TCT TTA AAG CGT TCG TGG TC	55.5 C	

The amplification reaction mixture (final volume, 25 µl) was carried out with thermal cycler (Techne, UK). The program of amplification reaction consisted of one denaturation step followed by 40 cycles and final elongation step described in table (3).

Amplification products were visualized in a 1.5% agarose (15), using gel electrophoresis stained with ethidium bromide. A Ladder 100 bp DNA was used as a size marker in the gels.

Table (3) PCR program.

No	Steps	Temperature	Time	Cycles
I	Initial Denaturation	94 °C	2 min	1
II	Denaturation	94 °C	30 sec	35
	Annealing	55 °C	30 sec	
	Extension	72 °C	45 sec	
III	Final Extension	72 °C	5 min	1

Statistical analysis

The results of present study were analyzed by SPSS program (version) software 2010, using Chi-square test and P values of $p < 0.05$ were considered to record statistical significance.

RESULTS

A- ELISA results

1- Result of ELISA According to animal's type

According to animals type of 387 sample of different animals, to detect *Toxoplasma gondii* ELISA results showed significant differences ($p<0.05$) different animals in presence of *toxoplasma gondii* IgG show in table (4). Sheep samples showed the highest positive results (33.036%) followed by goat and cattle (29.333%, 25.439% respectively), while the lowest positive results were showed in buffalo samples (11.628%).

Table (4) Distribution of *T. gondii* based on ELISA positive results in animals according to the type of animals.

Animals	ELISA Result		Total
	Negative	Positive (%)	
Cattle	85	29 (25.439%)	114
Sheep	75	37 (33.036%)	112
Goat	53	22 (29.333%)	75
Buffalo	76	10 (11.628%)	86
Total	289	98 (25.323%)	387
Chi square	13.970	df = 3	$p<0.05$

2-Result of ELISA According to samples type

The result of ELISA method made according to samples type of 387 different animals, the results showed significant differences ($p<0.05$) among serum, meat and minced meat as presented in table (5).

The highest positive ELISA results were showed in minced meat samples (37.113%), followed by meat samples collected from butcher markets (27.778%). while the blood and meat samples collected from slaughterhouse showed the same results (17.582%) which represent the lowest positive result.

Table (5) Distribution of *T. gondii* IgG results in animals according to the type of samples.

Sample	ELISA Result		Total
	Negative	Positive (%)	
Blood	75	16 (17.582%)	91
Meat after slaughtering	75	16 (17.582%)	91
Meat from butcher markets	78	30 (27.778%)	108
Minced Meat	61	36 (37.113%)	97
Total	289	98 (25.323%)	387
Chi square	13.136	df = 3	p<0.05

3-Result of ELISA According to site.

Recent results indicated that there were significant differences in seropositivity against *T. gondii* among the studied animals at different site in Basra city (p<0.05)-(Table 6). The highest positive results were detected in butcher markets (32.195%) while samples from slaughterhouse gave the lowest positive results (17.582%).

Table (6) The distribution of *T .gondii* seropositivity in animals of different regions of Basra city detected by ELISA.

Site	ELISA Result		Total
	Negative	Positive (%)	
Slaughterhouse	150	32 (17.582%)	182
Butchers Markets	139	66 (32.195%)	205
Total	289	98 (25.323%)	387
Chi square	10.127	df = 1	p<0.05

B- PCR Results

Toxoplasma gondii B1 replicon (133 bp) was detected in blood, meat and minced meat samples collected at slaughterhouse and butcher markets from different animals through PCR protocol (Figure1).

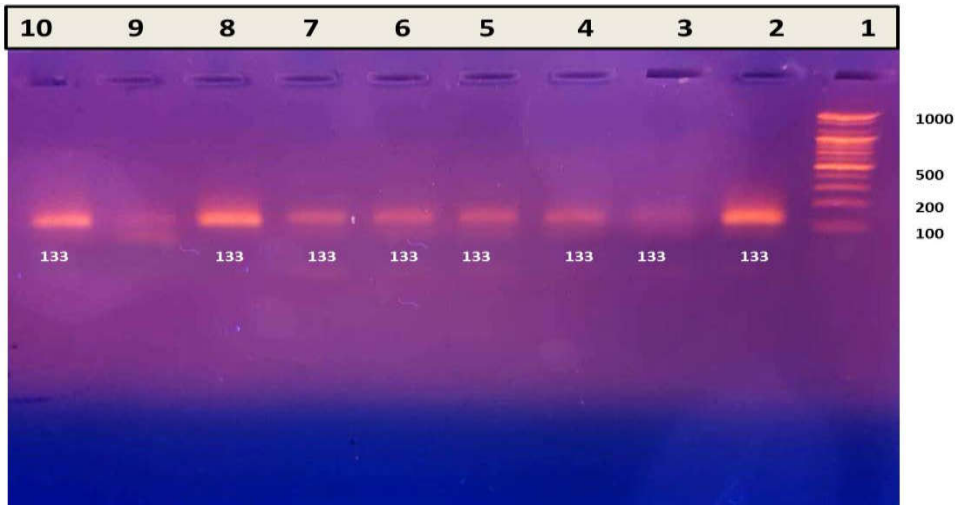

Figure 1 Agarose gel electrophoresis for *Toxoplasma gondii* B1 replicon Lane (2,3,4,5,6,7,8 and 10) represent the positive results while lane 9 negative, lane 1 indicates the Ladder 100 bp DNA marker.

1-Result of PCR According to animal's type

significant differences (p>0.05) in Toxoplasma gondii gene presence in different animals under study were detected through PCR protocol that applying to 387 of different animals type (Table7)

Sheep samples showed the highest PCR positive results (22.321%) followed by goat and cattle (18.667%, 14.912% respectively), while the lowest positive results were showed in buffalo samples (4.651%).

Table (7) Distribution of *T. gondii* according to the type of animals.

Animal	PCR Result		Total
	Negative	Positive (%)	
Cattle	97	17 (14.912)%	114
Sheep	87	25 (22.321%)	112
Goat	61	14 (18.667%)	75
Buffalo	82	4 (4.651%)	86
Total	327	60 (15.762%)	387
Chi square	14.345	df = 3	p<0.05

2- Result of PCR According to samples type.

According to samples type of 387 different animals, the PCR results showed significant differences (p<0.05) - between different samples (Table 8).

The lowest positive PCR results were showed in blood samples (6.593%) while the highest positive result were founded in minced meat samples (25.773%) fallowed by Meat from butcher markets and from slaughterhouse (18.519%, 9.89% respectively).

T**able (8)** Distribution of *T. gondii* according to the type of samples.

Sample	PCR Result		Total
	Negative	Positive (%)	
Blood	85	6 (6.593%)	91
Meat after slaughtering	82	9 (9.89%)	91
Meat from butcher markets	88	20 (18.519%)	108
Minced Meat	72	25 (25.773%)	97
Total	327	60 (15.762%)	387
Chi square	16.706	df =3	p<0.05

3- Result of PCR According to site.

There were significant differences in PCR results of *T. gondii* among the studied animals of different site in Basra city to detect *T. gondii* (p<0.05) that shown in table (9).

The highest positive results were detected in butcher markets (21.951%) while samples from slaughterhouse gives the lowest positive results (8.242%).

Table (9) The Distribution of *T.gondii* B1gene in animals from different regions of Basra city.

Site	PCR Result		Total
	Negative	Positive (%)	
Slaughterhouse	167	15 (8.242%)	182
Butchers Markets	160	45 (21.951%)	205
Total	327	60 (15.762%)	387
Chi square	12.805	df =1	p<0.05

Comparison of ELISA and PCR results

As a results of all data presented earlier there were significant differences ($p<0.05$) between ELISA and PCR results to detect *T. gondii* among the studied animals (Table 10).

The total positive results of ELISA test of *T. gondii* (25.323%) were higher than positive results of PCR test (15.762%) of overall sample tested.

Table (10) The comparison between ELISA and PCR protocols in detection of *T. gondii*.

Test		PCR Results		Total
		Negative	Positive (%)	
ELISA Results	Negative	289	0	289
	Positive (%)	38	60	98 (25.323%)
Total		327	60 (15.762%)	387
Chi square		204.757	df =1	p<0.05

DISCUSSION

Toxoplasma gondii is widely prevalent and one of the most widespread zoonosis in warm-blooded animals worldwide. *T. gondii* infection is widespread in some of the most consumed food containing meat, especially make from sheep, goat, cattle, buffalo, chicken, camel and pig. Eating undercooked or raw meat of animals and eating food or drinking water contaminated with oocysts are the important source of infection to human (16). The highest positive results for detection of T. gondii by ELISA and PCR in sheep samples in comparison with goat and cattle samples were in line with (5,17,16,18)who found that *T. gondii* is more frequently and consistently detected in sheep in comparison with goat and cow. The explanation of these differences may be refer to the geographical area, type of grassing and distribution of final host (cats), in addition to the natural susceptibility to infection of sheep by *T. gondii*. This in accordance with (19)who referred to that Goats are consuming the tops of grass and small trees which may have lower *T. gondii* contamination levels compared to sheep that are consuming the lower parts of the plants that *T. gondii* sero prevalence was reported to be higher level. About susceptibility (20) found that sheep were susceptible more 6 times than cattle.

However, Raw or undercooked goat meat identified as potential sources for T. gondii infection (21).

Result of ELISA and PCR According to animal's type

There were significant differences ($p< 0.05$) among animals type (Tables 4, 7). The positive of ELISA and PCR results in sheep samples (33.036%, 22.321% respectively) were the highest in comparison with goat (29.333%, 18.667%) and cattle (25.439%,14.912%) respectively. This finding were in line with (5,17,16,18)who found that *T. gondii* is more frequently and consistently detected in sheep in comparison with goat and cow.

Raw or undercooked goat meat identified as potential sources for *T. gondii* infection (21). Goats are susceptible to *T. gondii* infection, with reported seroprevalence of *T. gondii* infection in goats ranging from 3.7% (22) to 81.8% (23). *T. gondii* seroprevalence is generally lower in goats than sheep. The tops of grass and small trees are more consumed by goats which may have lower *T. gondii* contamination levels compared to sheep consumed the lower parts of the plants that *T. gondii* seroprevalence was reported to be higher level (19).

The explanation of these differences may be refer to the geographical area, type of grassing and distribution of final host (cats), in addition to the natural susceptibility to infection of sheep which is likely infected by *T. gondii* 6 times more than cattle (20).

On the other hand, the lowest result of buffaloes (11.628%, 4.651% respectively) may be due to buffaloes are considered resistant to toxoplasmosis and tissue cysts are reported to be found rarely in skeletal muscles of buffaloes **(**11).

Result of ELISA and PCR According to samples type

At recent research minced meat samples showed significant highest positive results for both ELISA and PCR method in comparison with meat from both butcher markets and slaughterhouse . The explanation of these finding may be due to the contamination of meat during grounding with other infected remaining meat at a contaminated mincer or equipment or due to mixing meat pieces from different carcasses at butcher markets.

These findings were in line with (24)who reported that ground meat considered as one of the main foodborne risk factor for the recent *T. gondii* infection in United States.

- On the other hand the positive ELISA results of sera samples obtained from animals before slaughtering gave the same results of meat samples obtained from the same animal after slaughtering, giving an idea that ELISA test can be used either in sera samples or from meat samples after slaughtering to detect *T. gondii* antibodies in eating animals. While the lowest positive PCR results were showed in blood samples which may explained by *T.gondii* is located predominantly in the central nervous system, as well as skeletal and cardiac muscle also in consistent with the other studies, Toxoplasma infection in various tissues (25)

Result of ELISA and PCR According to site

According to site that samples collect from, ELISA and PCR results in butcher markets in this study showed higher toxoplasmosis ratio compared with results from samples collected at slaughterhouse and this may due to the absence of monitoring in butcher markets compared with slaughterhouse. Regional variations may have been attributed to climate cultural **(**26).

Comparison between total results of ELISA and PCR

It is evident at present study that T. gondii B1 gene (133 base pair) was detected in blood, meat and minced meat samples collected at slaughterhouse and butcher markets from different animals by using primers pair 5'TTGCATAGGTTGCAGTCACT3' as foreword primer and 5'TCTTTAAAGCGTTCGTGGTC3' as reverse primer. According to table 10 the total 98 ELISA positive samples include 60 PCR positive samples. On the other hand there were no positive PCR samples in the 289 ELISA negative samples revealing that there where high correlation between ELISA and PCR results. While the lower significant ($p< 0.05$) positivity rate of PCR (15.762%) compared with the ELISA (25.323%) may due to the low concentration of cysts in the tissues and random distribution (one cyst per 50 – 100 g) of tissue and by the small size of samples **(**27).

Recommendations

1- Annual investigation of toxoplasmosis in domesticated animals including sheep, goats, cattle and buffalo.

2- The animals, meat and minced meat should be submitted for periodic investigation before and after slaughtering for detection of *T. gondii* as they might be toxoplasmosis carriers and hazardous for animals and human.

3- The slaughtering of animals should be done in slaughterhouses under veterinarian supervision.

4- Further studies should be done to investigate the prevalence of toxoplasmosis in imported meat and meat derivatives.

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