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Research Article

Detection of Toxoplasma gondii in blood and milk of infected goats and Pregnant women by Rapid test cassette and conventional -PCR methods in AL-Qadisiyah province, Iraq

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ABSTRACT

Aim: The study aimed to investigate the presence of the specific B1 gene T gondii in blood and milk samples from natural infected cattle and pregnant women (16-30 weeks) whose examination performed by the officers at the women's and children's Educational hospital in Al-Diwaniyah, Iraq.

Materials and methods: A total of 150 serum samplings were collected analysed and scanned for Anti-T gondi antibodies (75 naturally-infected goats and 75 pregnant women with Toxoplasma). Polymerase chain reaction (PCR) was used to detect of B1(399pb) gene in 26 goat's blood samples and 7 samples from pregnant women.

Results: A quick-test anti-cassette gondii results showed 26 positive samples of goats in a percentage of 34,666 percent, while a higher percentage of anti-T prevalence of 7 positive samples in women was 9,333 percent in age groups. T gondii antibodies in sera of goats were 56% in group 2-3 years in sera of goats, while in groups (20-25) years in women were 16%. Also, the higher prevalence of antibodies in goats and pregnant women was also 50% and 57.14% of IgG respectively.

PCR results showed that B1gene (399 bp) was detected in 33 blood samples (26 samples of goats and 7 samples from pregnant women). The present of B1 gene in 26 goat's blood samples was 38.46% and 57.14% of the 7 pregnant women's blood samples.

Conclusion: In total, 150 milk samples from the same group of goats and pregnant women have been obtained and analysed to investigate T gondii DNA. The amplified B1 gene was detected in 6 milk samples of 75 Goat samples (8%), whereas the findings showed that 75 milk samples of pregnant women did not contain the B1gene.

Keyword: Toxoplasma gondii, Goats, Pregnant women, Rapid test cassette, PCR-Technique

INTRODUCTION

Toxoplasmosis is one of the common diseases caused by a parasite known as the Toxoplasma gondii, an intracellular protozoan parasite accumulating in the nucleus of infected cells [1].

The main transmission of the disease can be occurred through the consumption of raw or partially cooked meat uncooked meal or through oocytes - contaminated containers and bags of food or living tissues. Moreover, blood transfusions are contributed to transmit the disease in the acute phase. In addition, this disease can infect bone marrow transplant recipients and cause fatal outcomes as they are on immunosuppressive treatments [2]. However, there are other alternative modes of transmission could be occurred for instance through inhalation of oocytes–contaminated dust or intake of unpasteurized milk [3], as well as the vertical transmission from mother to fetus through the placenta [4].

Toxoplasmosis has important concerns for both human and veterinary medicine. The organism is considered the main cause of infertility and abortion in humans and animals. In regards to the

public health, the infection has facilitated zoonotic transmission [5], through continuous contamination with T. gondii during sexual reproduction of T. gondii [6], that involves the release of Oocysts into the environment. Cats are important risk factor for T. gondii infection particularly during the sexual cycle and important factor the transmission of parasites to sheep and goats [7]. Previous studies concluded that this disease can be transmitted to human through the consumption of infected milk [8]

MATERIALS AND METHODS

Collection of blood samples

In the present study, a total number of 75 blood samples were taken from healthy female goats. The goats were from different regions of Qadisiyah province (Afak ,AL-Hamza ,Daghara) and there ages ranged from (1 – 4) years old between beginning of October 2017 to the end of July 2018. A volume of 5ml blood was collected from the jugular vein. Seventy-five blood samples were obtained from pregnant w omen (16-30 weeks) aged between (20-40) years, whose reviewers were split into two sections a t the Women's and Children's Hospital in Al-Diwaniyah.

The samples were divided into two groups; the first group was placed in EDTA free sterile test tubes for serological testing and the other section was placed in EDTA container tubes for polymerase chain reaction (PCR) testing, the samples were kept at fridge prior to sending to the college of science laboratory for analysis.

Collection of milk samples

Following the collection of blood samples from toxoplasma-infected goats and pregnant women, 5 ml of milk s amples were obtained. Prior to milk collection, iodine alcohol cleanses were undertaken to clean the goats teats and women's breasts. Milk was collected manually using protective gloves during the procedure (9). A sterile suction pump was used to collect milk from pregnant women. All milk samples were kept in sterile falcon tubes. The collecting tubes were labeled with number of animals and women and date and time of sample collection). Like the blood samples, milk samples were kept at fridge prior to sending to the laboratory in college of science for genetic analysis that involves DNA extraction and amplification using a PCR.

Serological examination

The blood samples were centrifuged at 300g for 15 min to separate the serum. The presence of anti-T. gondii antibodies (IgG & IgM) was examined using a rapid test cassette following the manufacturer's protocols.

DNA Extraction from blood and milk samples using the polymerase chain reaction (PCR)

The DNA was collected by using a special kit provid ed by the Bioneer company from the blood and mil k samples. PCR was performed by using a designed primer. A pair of primers for the B1gene (399bp), which was specific gene of T.gondii was designed by using (NCB1 Gene –Bank primer 3,No. AF179871.1 their destination by Bioneer company, Korea) as shown in table(1). The reaction mixture consists of the following components: Taq polymerase, dNTPs, Buffer PCR10x, $MgCl_2$, H_2O free nuclease and DNA template as indicated in table (2). The reaction mixture and Thermal cycler conditions are shown in table (3).

Table 1: The single nucleotide sequence of the nitrogen bases of the primers and size product of conventional PCR

Primers	Sequence	Product size
Forward primer	5-GAACCACCAAAAATCGGAGA-3	399bp
Reverse primer	5-GATCCTTTTGCACGGTTGTT-3	

Table 2: Components of the conventional PCR mixture

PCR master mix		Master mix volume (µl)
DNA template		5 µl
primers	F-primer	1.5 µl
	R-primer	1.5 µl

	PCR water	12 µl
Total		20 µl

Table 3: Polymerization chain reaction program for B1gene amplification

Steps	Temperature	time	cycle
Initial denaturation	95	5 min.	1
Denaturation	95	15 sec.	42
Annealing	65	25 sec.	
Extension	72	25 sec.	
Final Extension	72	1 min.	1

Electrophoresis

Using the power supply to outfit the 40 volt relay cell at theroom temperature for 1-3 hours and after the time has been finished a tool was used for the purpose of examining the position s of chromosome and plasmid using the UV radiati on source of 256 nanometres in length [10].DNA bundles were imaged on the Sony monito r.

Statistical analysis

The data were analyzed statistically using the statistical program) SPSS version 10.5 software). The X2- square was used to determine the significant differences below the probability level of $p \leq 0.05$. [11]

RESULTS

The study analysed blood samples using serological and molecular methods. The blood samples (n=75) obtained from female local goats from different areas of the province of Qadisiyah province (Afak, AL-Hamza, Daghara) and (n=75) collected from pregnant women.

The results indicated to the percentage of the prevalence of Anti-T. gondii antibodies in sera of goat was 34.666% (26 positive samples out of 75 samples) (table 4). Forty-eight percentage of Anti-T. gondii antibodies was found in sera of goat from Afak region (12 positive samples out of 25 samples), whereas only 20% of anti-T. gondii antibodies (5 positive samples out of 25 samples) was found in sera of goat from Daghara region.

Table 4: The prevalence of Anti-T. gondii antibodies in sera of goat in different regions of AL-Qadisiyah province by Rapid test cassette.

Regions	Examined. No	Infected No.	%
Afak	25	12	48*
AL-Hamza	25	9	36
Daghara	25	5	20
Total	75	26	34.666

*refer to significant from other at $P \leq 0.05$ א ^{2} calculated:2.165 א ^{2} tablets:2.920

Table (5) findings showed the greater proportion of the anti-T prevalence in sera of goats T gondii antibodies aged 2-3 years old, 56 per cent (14 positive samples out of 25) in the group, the lower 20 per cent (5 positive samples out of 25) in the group was 12 years old, with the higher prevalence of seropositive animals in goats 2-3 years old.

Table 5: The prevalence of Anti-T. gondii antibodies in sera of goat according to the age groups by the Rapid test cassette.

Age group	Examined. No	Infected No.	%
1-2 year	25	5	20
2-3 year	25	14	56*
3-4 year	25	7	28
Total	75	26	34.666

*refer to significant from other at $P \leq 0.05$ א ^{2} calculated:3.643א ^{2} tablets:2.920

AntiT.gondii antibodies in serum were shown to be 9,333 percent in pregnant women (7 positive samples of 75 samples) and 16 percent (4 positive samples of 25 samples); in the category of women aged 20-25 years as shown in table 6.

Table 6: The prevalence of Anti-T.gondii antibodies in sera of pregnant women according to the age groups by Rapid test cassette.

Age group	Examined. No	Infected No.	%
20-25	25	4	16*
26-30	25	1	4
31-40	25	2	8
Total	75	7	9.333

*refer to significant from other at P≤0.05 א ^{2} calculated: 1.797 א ^{2} tablets:2.920

Table findings (7) showed a higher prevalence of IgG immunoglobulin in goats (50 percent), while IgG was 57.14 percent in humans. The results of IgG T.gondii in goats and pregnant women also showed chronic. Moreover, the findings in table 7 showed that high levels of IgM in goats and pregnant women indicate acute infection.

Table 7: Types of Anti-T.gondii antibodies in sera of goats and pregnant women by Rapid test cassette

			Type of anti-T.gondii antibodies					
			IgG+IgM		IgG		IgM	
Type of sample	Examined. No	Infected No.	Infected No.	%	Infected No.	%	Infected No.	%
Goats	75	26	6	23.07	13	50	7	26.92
pregnant women	75	7	2	28.57	4	57.14	1	14.28

The T gondii infection of goats and pregnant women was confirmed using a PCR method. For this purpose, the B1 gene (399 bp) was detected in 26 blood samples from Goat and 7 blood samples from pregnant women, The findings showed that only 10 blood samples (%38.46) of goats were positive, whereas the B1 gene was only detected in 4 blood samples (57.14%) of pregnant women as shown in table (8), figure (1)and (2).

Table 8: Prevalence of B_1 gene (399bp) of T.gondii in blood samples of goats & pregnant women by conventional –PCR.

Type of sample	Examined. No	Positive number (%)	Negative number (%)
Goats	26	10 (38.46%)	16 (61.53%)
pregnant women	7	4 (57.14%)*	3 (42.85%)
Total	33	14 (42.42%)	19 (57.57%)

*refer to significant from other at P≤0.05 א ^{2} calculated: 1.618 א ^{2} tablets:6.314

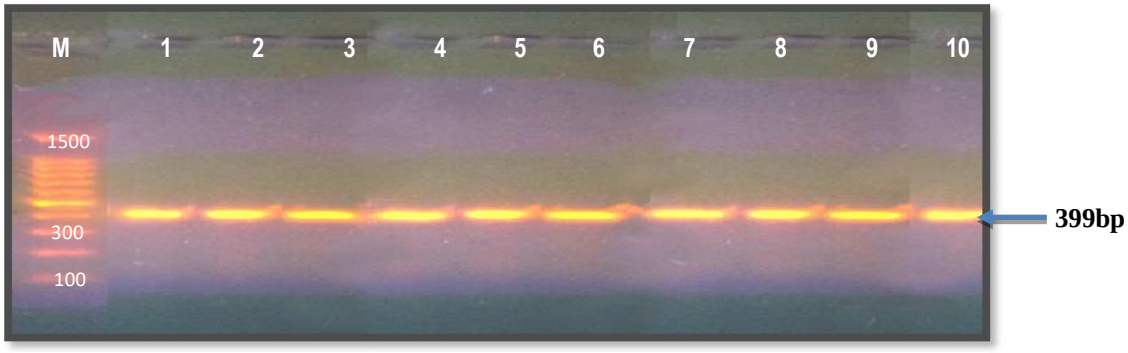

Fig.1: Agarose gel electrophoresis image that show the PCR product analysis of B1 gene in blood samples positive in goats. Where M: marker (100-15000bp), lane (1, 2, 3, 5, 8 and 10) positive *T.gondii* at (399bp) PCR product size.

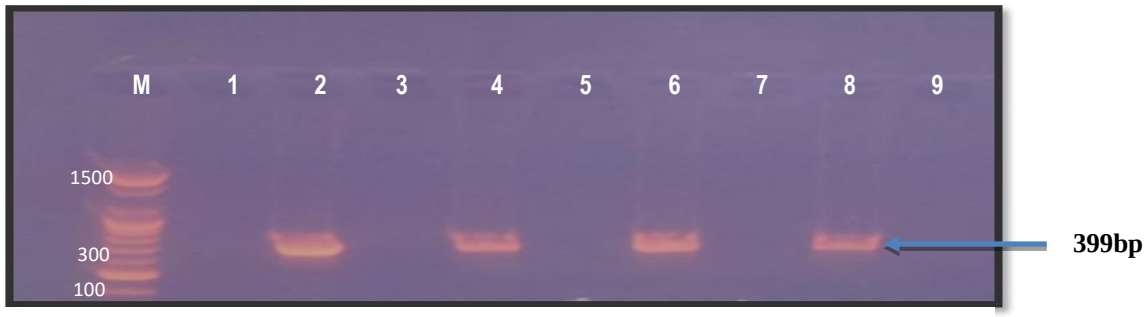

Fig.2: Agarose gel electrophoresis image that show the PCR product analysis of B1 gene in blood samples positive in women. Where M: marker (100-15000bp), lane (2, 4, 6, 8) positive *T.gondii* at (399bp) PCR product size.

The tests of B1 gene amps (399bp) were tested in 75milk samples obtained from each of goats and pregnant women. The results showed positive for PCR by percentage (8%) in 75 samples of the milk of the goat as shown in table (9), whereas no B1 gene was detected in 75 milk samples of pregnant women as shown in table (9), figure 3 and 4.

Table 9: Prevalence of B_1 gene (399bp) of T.gondii in milk samples of goats & pregnant women by conventional –PCR.

Type of sample	Examined .No	Positive number (%)	Negative number (%)
Goats	75	6 (8%)*	69 (92%)
pregnant women	75	0 (0%)	75 (100%)
Total	150	6 (4%)	144 (96%)

*refer to significant from other at P≤0.05 א ^{2} calculated: 5. 67 א ^{2} tablets: 6.314

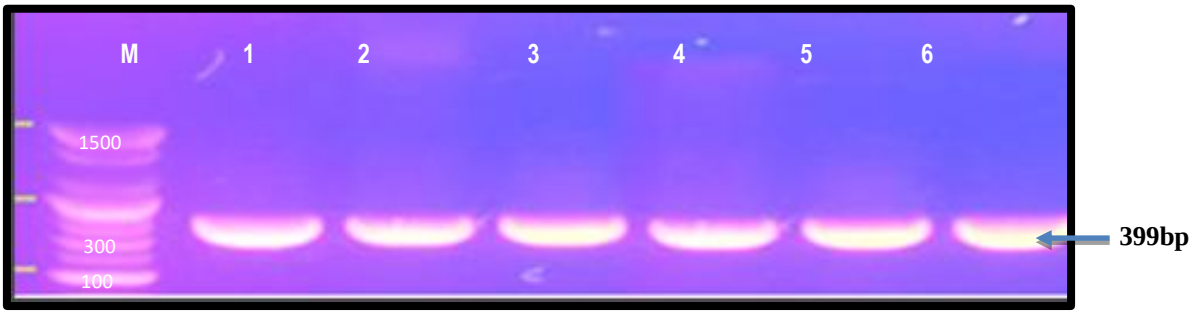

Fig. 3: Agarose gel electrophoresis image that show the PCR product analysis of B1 gene in milk samples positive in goats Where M: marker (100-15000bp), lane (1,2, 3, 4, 5 , 6) positive *T.gondii* at (399bp) PCR product size.

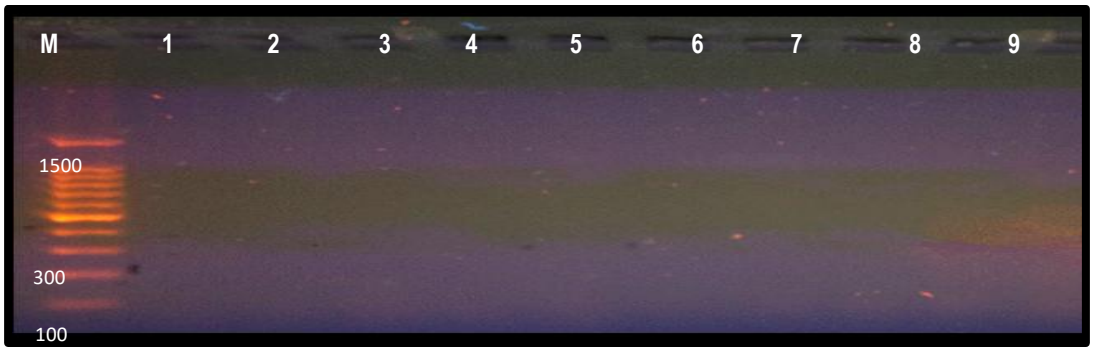

Fig. 4: Agarose gel electrophoresis image that show the B1 gene was not present in 75 milk samples of pregnant women Where M: marker (100-15000bp), lane (1-10) negative *T.gondii* at (399bp) PCR product size.

DISCUSSION

In many economies, cattle are substantial source of for milk and meat production, however, because of the extent of the transmission of T.gondii by unpasteurized milk from goats to human, the goats and pregnant women have been targeted for this study.

The study analysed the sera of goats in Afak and Daghara regions and found that higher percentages (48%) of anti-T. gondii antibodies in the former and only 20% of anti-T. gondii antibodies in Daghara region. The presence of the higher distribution of T. gondii in Afak region could be resulted from the environmental conditions in this area including, humidity and warm climates in, these reasons could be contributed to the distribution of T.gonddi in goats in the region of Afak.

The greater proportion of the anti-T prevalence in sera of goats, T gondii antibodies, was found in aged 2-3 years old, 56 %, the lower 20% detected in the sera of goats aged 12 years old, with the higher prevalence of seropositive animals in goats 2-3 years old. This could be attributed to the care of these animals and management activity of these animals and the feeding habits of them may be related to the 2-3 years old community of animals directly on land and rudimentary (12).

The anti T.gondii antibodies in serum of pregnant women was higher at age 20-25 (16%) and decreased by half at aged 31-40 years(8%) . The latter age group expressed the double of antibodies in sera compared to 26-30 age group(4%). The findings suggested the discrepancies among the three age groups that are prone to decrease with ageing progress.

High prevalence of IgG + IgM formation in goats and pregnant women indicated a chronic infection. This finding was confirmed previously by (13). The expression of B1 gene was tested in blood and milk samples of goats and pregnant women.

Examinations showed that higher percentages of B1 gene was detected in the samples of blood and

milk obtained from goats compared to pregnant women. There is no B1 gene detected in the milk samples of pregnant women.

The PCR was used to scan the agent in milk as a good indicator of the existence of the parasite in the material examined, although the existence of DNA does not indicate the viable nature or possible infectiousness of the agent [14].

Several studies have shown that T.Gondii infected milk intake may cause human disease [15] and tachyzoites in various species 'milk such as sheep, goats, camels, and bovine animals [16, 17] and T.Gondii tachyzoite may be excreted into milk while being acutely infected in goats and become a potential source of human infection)[18,19].

CONCLUSION

The findings of this study demonstrated that T gondii DNA can be transmitted in the blood of goats and pregnant women and in goat's milk, only through ingestion of raw goat milk. Since goats are the largest milk sources in the entire Iraq region, the host may be highly contaminated by raw milk.

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