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Original Article

Molecular and Seroprevalence of Toxoplasmosis in Goats' Blood and Milk in Iraq

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Abstract

Toxoplasmosis is one of the most acute parasitic and zoonotic infections, which causes severe economic losses in animals due to abortion and reproductive problems worldwide. Therefore, this study was conducted in Baghdad province to detect the prevalence of Toxoplasmosis in blood and milk samples of 384 adult female goats using the serological indirect-enzyme-linked immunosorbent assay (iELISA) and the molecular polymerase chain reaction (PCR) test. The positive iELISA results were 20.57% in sera and 5.99% in milk samples. Regarding cross-classification results, the iELISA results revealed that 5.73% of goats were positive by testing both sera and milk samples, 14.32% and 0.26% were positive for testing sera and milk only, respectively, and 79.69% were negative by testing sera and milk. Targeting the B1 gene, the total positive results of the PCR assay showed that 13.92% and 30.43% of blood and milk samples, respectively, were positive at 546bp. Concerning cross-classification results, the total positive goats by testing of both sera and milk was 8.86%, while 5.06% of goats were positive only for testing of blood, and 86.08% were negative for testing of both samples. At the same time, no positive PCR results were detected in milk samples. In conclusion, there is a wide prevalence and incidence of Toxoplasmosis among goats in study areas. Furthermore, studies are essential to detect the parasite in different ages and sexes of goats and other domestic and wild animals using ELISA as a reliable, automated, and rapid test and PCR as a highly confirmative test.

Keywords: *Toxoplasma gondii*, Caprine, ELISA, PCR, Iraq

1. Introduction

Toxoplasma gondii, an opportunistic protozoan parasite of the Apicomplexa phylum, was first detected by Nicolle and Manceaux in 1908 and later had more attendance due to its great medical and veterinary importance (1, 2). Although the genus is comprised of a single species (*T. gondii*) that exists in one definitive host (Felidae), it infects most domestic and wild mammals as intermediate hosts worldwide (3, 4). Also, the wider genetic diversity of atypical and non-clonal strains is identified among most countries in Asia, Africa, Europe, and South America (5). The infection can generally lead to several economic losses, especially in herds, due to abortion, stillbirth, pneumonia, and changes in susceptible goats' digestive and neural systems (6). In addition, therapeutic approaches for Toxoplasmosis have not developed well for many years since no drugs could eliminate infection (7, 8). The parasite can transmit naturally from the final definitive host to the accidental intermediate hosts or vice versa and between the various intermediate hosts (9). Domestic animals like goats and sheep are the primary source of transmitting the infection to humans through eating under- / un-cooked meat or drinking

unpasteurized milk (10). However, a goat appears to be highly susceptible to clinical Toxoplasmosis that may result in abortion and neonatal or adult mortalities during the acute phase of infection (11).

Several laboratory methods were developed and modified for detecting the prevalence of infection and diagnosis of hosts, which are based on the identification of specific antibodies. The factual investigation of Toxoplasmosis should rely on reliable serological data to drawback false-negative and/or false-positive results (12, 13). Using the polymer chain reaction, the parasite DNA is amplified and its genotypic diversity was determined in humans or animals (14).

In Iraq, despite Toxoplasmosis being well studied in cattle and sheep, information about the prevalence of goat toxoplasmosis or infection epidemiology is low and not exhaustively detailed. In addition, the lack of data dealing with caprine milk as a potential source for Toxoplasmosis justifies the importance of this study. The present study was carried out to estimate the prevalence of goat IgG anti-*T.gondii* antibodies in both blood and milk samples by applying an ELISA test and to demonstrate the existence of parasites (or their DNA) in blood and milk samples by a molecular PCR technique.

2. Materials and Methods

2.1. Samples Collection

In many areas related to Baghdad province, a total of 384 adult female goats (≥ 1.5 years) selected randomLy were submitted for this study from February to July 2021. After dipping the udder, approximately 50 mL of milk was collected into a disposable plastic container. Each milk sample was divided into 10 mL for molecular testing and 40 mL for the ELISA test. Then, 5 mL of venous blood was drained from each animal by a disposable syringe and allocated equally into without and- anticoagulant EDTA tubes to be used later for serology and molecular assay, respectively. Both milk and blood samples prepared for serology were centrifuged (4000 rpm for 15 minutes). Sera of blood was kept in labeled Eppendorf tubes and frozen, whereas the supernatant of milk under the fat layer was pipetted into new tubes to be re-centrifuged. The clear supernatant was pipetted into Eppendorf tubes and stored frozen at -20°C until tested (15).

2.2. Serological Examination of Blood and Milk Samples

The controls, reagents, and samples were made and tested according to the TOXO IgG-antibody iELISA Kit (Aviva System Biology Company, USA) instructions. The optical density (OD) was measured at 450 nm using an ELISA-reader system (BioTech, USA).

2.3. DNA Detection in Blood and Milk Samples

All positive blood and milk samples by ELISA were tested by PCR assay. The DNA were extracted using the QIAamp DNA Minikit (Qiagen, Germany). The purity and concentration of DNA samples were evaluated using the Nanodrop system. As described previously (16, 17), specific primers (Bioneer, Korea) were used for amplifying fragments of the *T. gondii* B1 gene: Toxo B22 (F) (5'AACGGGCGAGTGAGCACCTGAGGAG'3) and Toxo B23 (R) (5'TGGGTCTACGTCGATGGCATGACAAC'3) with 546 bp. The PCR reaction was carried out in a mixture of 1× PCR buffer, 2 mM MgCl2, 10 µM of each primer, 0.2 mM of each dNTP, 2 U Taq polymerase, 1.5 µl of DNA template, and distilled water to a total volume of 25 µl.

All PCR reactions were carried out by using a thermal cycler (MJ-BIO RAD, USA), recombinant Taq DNA (GENET-BIO, Korea), and initiated by one cycle (95°C/5 minutes), 40 cycles each for denaturation (94°C/30 seconds), annealing (60°C/30 seconds), extension (72°C/1 minute), and termination by a final extension (72°C/10 minutes). The amplification products were detected by using 1.2% agarose gel-electrophoresis that was visualized with 0.5% ethidium bromide in the persistence of ultraviolet light.

2.4. Statistical Analysis

All obtained data were introduced and analyzed with two programs, Microsoft Office Excel (2013) and

IBM/SPSS (*V.23*), by using the descriptive statistics and Chi-square test (X^2). The differences in the blood and milk samples or PCR results were considered significant at values of $P \leq 0.05$ (20).

3. Results

The iELISA was used to do a serological test on 384 goat serum samples and 384 goat milk samples. Table 1 shows that 79 (20.57%) of the serum samples and 23 (5.99%) of the milk samples were positive.

Table 1. Seroprevalence of *T. gondi* in blood and milk samples by indirect-ELISA

Sample		Total No.	Seropositives	Seronegative
1	Sera	384	79 (20.57%)[a]	305 (79.43%)
2	Milk	384	23 (5.99%)[b]	361 (94.01%)

When sera and milk samples had been tested by an indirect ELISA, the study's cross-classification results revealed that 22 goats (5.73%) were positive for both samples and 306 goats (79.69%) were negative for both samples, while 55 goats (14.32%) were positive for sera only and 1 goat (0.26%) was positive for milk only (Table 2).

Table 2. Cross-classification indirect-ELISA results among blood and milk samples

	Milk Results		Total
Sera Results	**Positive**	**Negative**	
Positive	22 (5.73%)[Ba]	55 (14.32%)[Ab]	77
Negative	1 (0.26%)[Bb]	306 (79.69%)[Aa]	307
Total	23	361	384

Variation in large horizontal and small vertical letters refers to significance * ($P \leq 0.05$)

A molecular PCR technique examined a total of 69 seropositive blood and 23 seropositive milk samples; the results reported that 11 (13.92%) blood and 7 (30.43%) milk samples were positive (Table 3).

Table 3. PCR results among blood and milk seropositive samples

Sample		Total No.	positives	negatives
1	Sera	79	11 (13.92%) [b]	68 (86.08%)
2	Milk	23	7 (30.43%) [a]	16 (69.57%)

Amplification of PCR products for external and internal genes of positive *T. gondii* isolates was visualized at 546 base pairs (bp), respectively, with 1.2% agarose gel-electrophoresis as follows: in (Figure 1) Lane (M) represented the DNA marker (100-1500 bp), and the Lanes (1-11) were the positive blood samples; whereas in (Figure 2), Lane (M) represented the DNA marker (100-1500 bp), and the Lanes (1-7) were the positive milk samples.

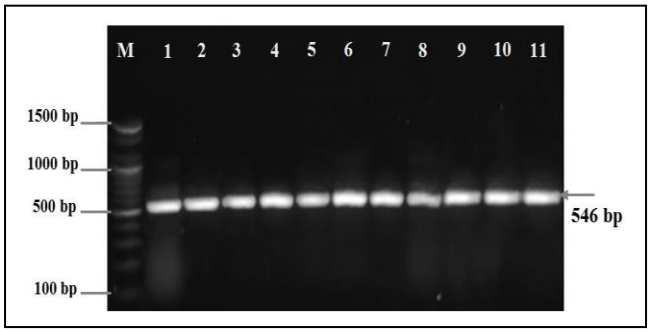

Figure 1. Amplified DNA of positive PCR products among blood samples

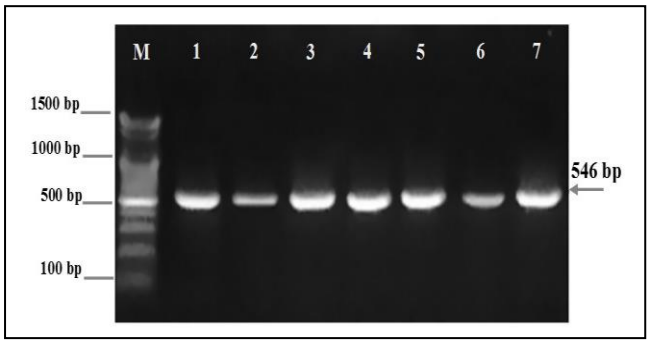

Figure 2. Amplified DNA of positive PCR products among milk samples

Among cross-classification results for blood and milk samples tested by PCR technique, the study showed that 7 (8.86%) of goats were positives with both samples, and 68 (86.08%) of goats were negatived by both samples; whereas 4 (5.06%) of goats were positives by blood and negatives by milk examination (Table 4).

Table 4. Cross-classification PCR results among blood and milk samples

Sera Results	Milk Results		Total
	Positive	Negative	
Positive	7 (8.86%)^{Ba}	4 (5.06%)^{Bb}	11
Negative	0 (0%)^{Bb}	68 (86.08%)^{Aa}	68
Total	7	72	79

Variation in large horizontal and small vertical letters refers to significance * ($P \leq 0.05$)

4. Discussion

Toxoplasmosis is a zoonotic infection caused by *Toxoplasma gondii*; the presence and survival of *T. gondii* tachyzoites in goat milk makes it a source of Toxoplasma infection. In Iraq, the present study was the first to detect *T. gondii* in blood and milk samples of goats using indirect-ELISA and PCR techniques. The exposure of goats to *T. gondii* in studied areas might be widespread because the overall seroprevalence of specific IgG-antibodies was 20.57% and 5.99% in examined sera and milk samples, respectively, by using a serological indirect-ELISA. It might mean that the goats are either infected or exposed to sources of toxoplasmosis infections from the contaminated pasture or other domestic or wild animals (18). The cross-classification results of blood and milk samples reported that 22 of 23 seropositive milk samples were also seropositive through examining of blood samples, whereas 55 positive sera samples were detected to be negative by testing of milk because of either the high levels of circulating IgG antibodies in blood compared to milk, or the presence of some milk proteins that could hamper the test antibody-antigen reaction (19). The prevalence of goat toxoplasmosis could vary among regions, diagnosis, and cut-off point (20). However, different data has been obtained on the prevalence of *T. gondii* in goats. However, the seroprevalence of goat *T. gondii* was 12% (21), 21.3% (22) and 47.4% (23) in Iraq; 25.1% in Brazil (24), 39% in Egypt (25), 22.7% in Iran (26), 11.4% in Myanmar (27), 17-75% in Norway (28), 60% in Portugal (29) and 32.5% in Tunis (30). The variability in seroprevalence of specific *T. gondii* antibodies might have biological and epidemiological explanations such as differences in the age of animals, environmental lifestyle, an abundance of oocysts in the soil, and hygiene standards of the farms (31, 32). Although the increase in goat toxoplasmosis prevalence might be attributed to the high persistence of cats in the field, mixed grazing with other domestic animals, climate, contamination, or variation in genetic makeup (30, 33), the low prevalence could be explained by the low exposure to infective oocysts in their environment (34).

Using the PCR technique, the prevalence of *T. gondii* DNA in seropositive blood and milk samples was 13.92% and 30.43%, respectively, which demonstrated the possibility of toxoplasmosis transmission by milk. Despite the fact that the seroprevalence rate of toxoplasmosis infection must be higher than the PCR result (26) and the molecular results of milk should be less than that of blood (35); the results of this study reported that the PCR positive milk data were higher than those detected in seropositive milk samples as well as in positive PCR blood samples ($P>0.05$), This may be due to the small samples of positive milk tested on infected goats (15). Nonetheless, the cross-classification PCR results of blood and milk samples were significantly similar ($P \leq 0.05$).

Brain and/or skeletal muscles were used to accomplish the PCR technique (36). However, the prevalence of Toxoplasmosis by PCR was 6% in Brazil (16), 16% in Egypt (25), 7.9% in Italy (35), and 32.5% in Tunis (37). As field animals, goats become infected with this protozoan when they eat grain, grass, or hay contaminated with cat feces. They then enter the small intestine to nearby lymph nodes to spread throughout the body tissues and/or organs for years (38). Mammary cells could be harbored *T. gondii* cysts acting as silent cysts during the pre-lactation period and transmitting with milk after suckling trauma or tissue cyst excretion (39). Also, the silent cyst could be secreted from the mammary gland cells by exocytosis and coated by host-cell membranes (as milk fat globules secretion), resulting in insidious and inner

contamination of milk (25). The transmission of *T. gondii* tachyzoites by raw milk was documented in other field animals such as cattle and sheep (40, 41).

The obtained data showed a significant prevalence of Toxoplasmosis in blood and milk samples. The lack of positive milk samples by PCR suggests that the parasite could not transmit by the milk of positive goats, or shedding could occur intermittently. In addition, the positive Toxoplasmosis in goats could be a source of infection for their environments and surrounding animals. Further investigation in other areas must be established using iELISA as a reliable, automated, and rapid test and PCR as a high confirmative test.

Authors' Contribution

Study concept and design: H. A. J. G.
Acquisition of data: H. A. J. G.
Analysis and interpretation of data: M. A. R.
Drafting of the manuscript: M. A. A.
Critical revision of the manuscript for important intellectual content: M. A. R.
Statistical analysis: M. A. A.
Administrative, technical, and material support: M. A. R.

Ethics

This study was licensed by the Scientific Committees of the College of Veterinary Medicine (the University of Baghdad, University of Wasit) and the Dijla University College.

Conflict of Interest

The authors declare that they have no conflict of interest.

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