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RESEARCH ARTICLE

Molecular Detection of *Toxoplasma gondii* in Human and Chicken by Real-Time PCR Technique

*** Huda Abd Al-Hadei Al-nasrawi, Hassan Hachim Naser, Saba Falah Kleaf**
Zoonotic Diseases Research Unit. College of Veterinary Medicine, University of Al-Qadissiya

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Corresponding Author

Baha'a A. Abdul-Hussein

Abstract

The Toxoplasma gondii is one of the most common zoonotic infectious pathogenic parasites and can be infected the human and animals. Regarding to transmission of disease by feline, the chicken can be a risk factors for Toxoplasma infection by transmission the oocyct. Current diagnosis of toxoplasmosis dependent on serologicaldetectionit may fail todetect specific anti-Toxoplasma IgG orIgM during the active phase of T. gondii infection, becausethese antibodies may not be produced until after several weeksof parasitemia. Therefore, in this study we used highly specific molecular as Real-Time PCR based TaqMan probe and primers to amplify the T. gondii B1 gene for detection of Toxoplasma gondii. Our results recorded the detection of Toxoplasma gondii in positive blood samples by LAT test collected from aborts woman and local breed chicken at (16%) and (24%) respectively. We conclude that real-time PCR a rapid, sensitive molecular technique specific for detection of T. gondii in human, animals and the chicken may be play important role in transmission of disease. Using the Real-Time PCR technique as detection tools of toxoplasma gondii in human and chicken was reported at first time in Iraq

Introduction

Toxoplasmosis is a common parasitic disease caused by the protozoan parasite Toxoplasma gondii**.** Toxoplasmosis is a zoonosis of worldwide distribution caused by Toxoplasma gondii, an obligate intracellular coccidian parasite that infects most warm-blooded animals including birds, humans, domestic and wild animals (7).Birds can be considered important reservoirs of T. gondii as they are often hunted by felids; they proliferate out of control as they are not selective about food, eating food waste that may be contaminated with T. gondii. In addition, as they fly long distances and can feed on the ground, they can be potential hosts of this coccidian (11).

The diagnosis of T. gondii infection by direct detection of parasite-specific DNA in biological samples using PCR-based molecular methods has gained popularity. The molecular diagnosis is more sensitive and cost-effective than the conventional methods (4).Molecular methods rely on PCR for the specific detection or analysis of T. gondii DNA. These methods have proved to be simple, sensitive, reproducible and cost-effective, and have been applied to a variety of clinical samples from animals and humans (2, 5, 3, and 1).Real-time PCR has been used to amplify and quantify DNA from the T. gondii B1 gene (6, 8). This quantification of parasite DNA can be used to determine the number of parasites in tissues and fluids, such as the amniotic fluid of patients suspected of being congenitally infected with T. gondii., The real-time PCR is a highly sensitive and specific method, however it is expensive and requires specialized detection systems and therefore may only be cost-effective in laboratories where analysis of large numbers of samples is carried out (9).Real-time PCR results from peripheral blood samples originating frompatients suspected of acute toxoplasmosis according to the serological criteria for acuteinfection, i.e. avidity of specific IgG antibodies and the finding of specific IgM antibodies. (10).

Materials and Methods

Samples collection: blood samples from aborts woman were collected from Al-Mothana hospital laboratory andblood samples from local breed chickens were collected different farmsin Iraq,two blood samples from each individual human and chicken.The collectedblood samples from each bird were kept in clean and sterilized test tubes without anticoagulant for serumcollection and with anticoagulant for DNAextraction and transported in to the laboratory.Clotted blood samples were kept in arefrigerator overnight. Serum was separated bycentrifugation at 3000 rpm for 15 minutes and stored at –20 °C till examination.Another non-clotted blood samples were stored in -20°C refrigerator until use for genomic DNA extraction.

Latex agglutination test (LAT)

Sera were examined usingthe latex agglutination slide test (LA) (SGM
Italia, Roma, Italy) as described earlier (13). Thetiter 1:8 or higher was considered as positive.

Genomic DNA extraction: Genomic DNA was extracted from frozen blood samples by using (Genomic DNA mini Extraction kit. Geneaid. USA). 200µL blood placed in 1.5 ml microcentrifugeand 20µL proteinase K (10mg/mL) was added for cell lysis. Then genomic DNA extracted according to kit instructions. The purified DNA was eluted in elution buffer provided with kit and store at -20°C, and the extracted DNA was checked by Nanodrop spectrophotometer.

Real-Time PCR

Real-Time PCR basedTaqMan probe was performed for rapid detection of T. gondiiaccording to method described by Meihuilinet al. (11). Real-Time PCR TaqMan probe and primers were used for amplification of conserved region B1 gene in T. gondii .These primers were provided by (Bioneer Company. Korea) as showed in following table (1):

The Real-Time PCR amplification reaction was done by using (AccuPower® DualStar™ qPCR PreMixBioneer. Korea) and the qPCR master mix were prepared for each sample according to company instruction as following table (2):

Table (2):The qPCR master mix

These qPCR master mix reaction components that mentioned in table above were added into AccuPower® DualStar™ qPCR PreMixtubes which containing Taq DNA polymerases, dNTPs, 10X buffer for TaqMan probe amplification. Then tubes placed Exispin vortex centrifuge at 3000rpm for 3 minutes, after that transferred into MiniOpticon Real-Time PCR system and applied the following thermocycler conditions as the following table (3):

Table (3): Thermocycler conditions

Results and Discussion

Toxoplasmosis encompasses a large variety of hosts including human, animals and birds ,the main sources of infecting humans, is bird meat, so besides another indicators to detect the distribution of T.gondiioocysts in the environment, the determination of T.gondiiprevalence in domestic birds is of great importance(12).

The Results of this study proved the occurrence ofconsiderable percentages of T. gondii infection in aborts woman and local breed chickens.The confirmed positive samples to latexagglutination test by real-time PCR technique. The 50 positive bloodsamples by LAT test collected from the aborts woman showed only 8 positive samples by Real-Time PCR technique based TaqMan probe for detection of Toxoplasma gondiiwhichrepresented (16%); whereas, the 50 positive blood samplesby LAT test collected from local breed chicken showed only 12samples being positive byReal-Time PCR technique, (24%),Theseresults agree witha number ofstudies has already shown that a positive PCR result is not always accompanied by positiveserology indicating local synthesis of antibodies (14,15)

The positive samples in Real-Time PCR which appear in threshold cycle (C_T value) ranged from C_T: 25 to C_T: 36 as show in (Figure-1).

Table (1): Real-Time PCR TaqMan probe and primers

Primer	Sequence	
B1 gene Primers	F	TCCCCTCTGCTGGCGAAAAGT
	R	AGCGTTCGTGGTCAACTATCGATTG
B1 gene	FAM-TCTGTGCAACTTTGGTGTATTCGCAG-TAMRA	

Probe	

qPCR master mix	Volume
Genomic DNA template	5µL
B1 Forward primer (20pmol)	1µL
B1 Reverse primer (20pmol)	1µL
B1 TaqMan probe (25pmol)	2 µL
DEPC water	11µL
Total volume	20µL

qPCR step	Temperature	Time	Repeat cycle
Initial Denaturation	95 ℃	5 minute	1
Denaturation	95 ℃	15 sec	45
Annealing\ Extension	60 ℃	1 minute	
Detection(scan)			

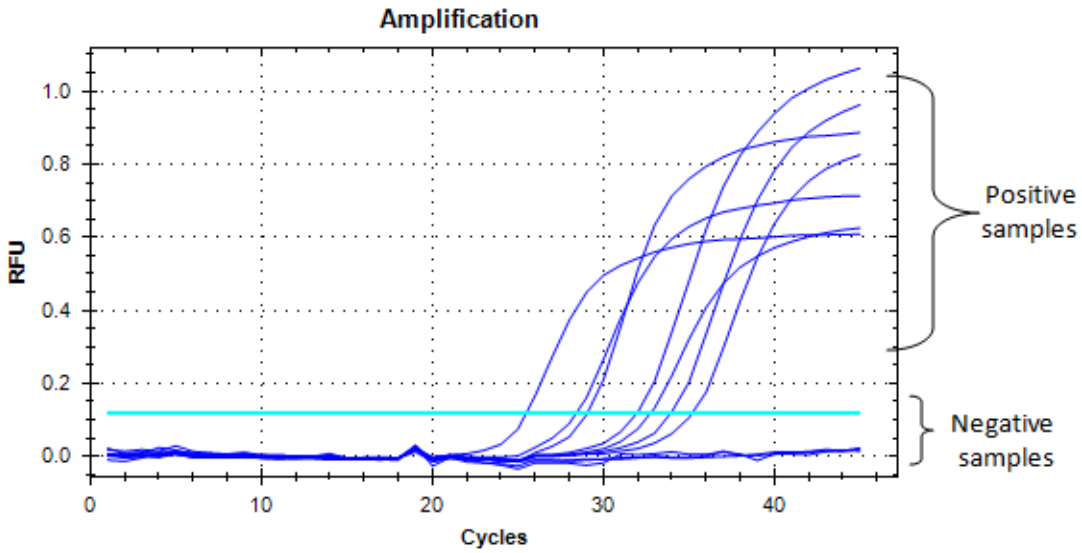

Fig (1): Real-time PCR amplification plot of B1 gene T. gandii from blood samples of human and chicken source.

Molecular detection of Toxoplasma gondii by Real-Time PCR was appeared specific and highly sensitive assay, this results was consistent with (2) who developed a rapid, sensitive, and quantitative real-time PCR for detection of T. gondii from different clinical specimens. Therefore, this technique has advantages rapid molecular tools for diagnosis of toxoplasmosis in a clinical laboratory, rather than serological test.

These results included public health significance,especially through consumption of undercooked poultrymeat. It can be certain on that PCR technique isconsidered a sensitive tool for the diagnosis oftoxoplasmosis where a

positive results can be counted on asan early marker for reactivations. Also, this techniqueproved to be a powerful tool for monitoring therapy.

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