Molecular Detection of *Toxoplasma gondii* in *Helix pomatia* in Wasit Province Iraq

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Abstract

DNA of a total of 96 samples of (32) enteric, (32) gastric and (32) pod of *Helix pomatia* has been obtained through the use of the Genomic DNA Extraction kit and tested by the use of the Real time PCR and specific set of the primers and probe for the detection of the *Toxoplasma gondii* B1 gene. Six enteric samples and only 2 gastric samples have been positive while the others have been negative, that result indicates the presence of the *T. gondii* in that snail species, and based upon those results infection can either be acquired from the contaminated environment or as a result of consuming the grass that has been contaminated with parasite oocysts. The negative result of pod samples may be due to the failure of filtration the infectious stages of parasites into the organs that close contacts with environment or inability of the parasites in surviving in those organs.

Key-words: *Toxoplasma Helix IRAQ real time PCR*

Introduction

The protozoan *Toxoplasma gondii* can be described as an obligatory microscopic parasite, it is avery common eukaryotic pathogens worldwide, cause Toxoplasmosis that infects most warm-blooded animals including birds, humans, domestic and other terrestrial animals [(1)]. Wild and domestic cats and other felids play as final hosts shedding oocysts in their feces; the infection can be acquired by the exhaustion of the undercooked or raw meat that contains tissue cysts, from soil, food or water that has been contaminated by the oocysts; or contact with infected carcasses during evisceration, processing or dressing, handling of the organs and other tissues of game animals, which risk of infection to humans [(2, 3, 4)]**.** The toxoplasmosis infection produces the generalized parasitemia involvements of liver, lungs, brain as well as other organs, and usually results in death [(1, 5, 6, 7)]. The ability of the oocyst to resist fluctuating environmental conditions such as dryness, freezing and sunlight, which is excreted with cat litter. It makes it a permanent and available source of infection [(8, 9)]. The *Toxoplasma gondii* parasite is characterized by its great ability to move in the water, so oocysts can remain able to infect in the water and soil for several months or several years [(10, 11)]. The 1st explosion of the *T. gondii* in the marine mammal has been designated by Dietrich and Van Pelt in the year of 1973 in harbor seal [(12, 13, 14)]. The infection with the *Toxoplasma* parasite took another turn that is not limited to marine and terrestrial mammals, as the infection acquired other types of invertebrates, including aquatic mollusks, as the parasite eggs were detected by microscopic examination of feces and snail tissues 10 days after exposure to the parasite oocyst [(15)]. Aquatic invertebrates can act as primary hosts through the accumulation of *T.gondii* eggs and their concentration in their tissues, so transporting food can be an important pathway for the transmission of *T. gondii* oocyst. Where the ability of abalone to swallow and accumulate *T. gondii* oocyst was proven in its tissues and concentrated, as *T.gondii* DNA has been detected in its tissues following the exposures to the parasite oocyst [(16)]. In Italy, Putignani *et al.,* (2011) [(17)] obtained in their investigations of *T. gondii* in oysters, using Real time and Nested PCR, a positive rate of 6.6% of the total number of samples examined.

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Esmerini *et al.,* (2010) [18] and Miller *et al.,* (2008) [19] have identified the naturally acquired *T. gondii* in the mussel through the use of the multi-locus PCR and DNA sequencing, and as well by nested PCR targeting B1gene locus in 3.30% of a 300 samples of the oysters. The bivalves'capacity in acquiring the pathogens from the sea water has been well known [18, 21]. The analyses have shown as well that *T. gondii* holds the responsibility of the high rates of the mortality in the otters in some California coastareas, and this percentage has been related to consuming infected marine snails [3, 22]. Sea otters which preyon the marine snails have an 11-fold increase in the *T. gondii* infection risk, while the predation on the bivalves has not been one of the considerable factors of risk [23]. In the case of the natural conditions, *T.gondii* has been found lately in the wild shellfish like the *Crassostrea rhizophorae* and *Mytella guyanensis* in Brazil [18] and *Mytilus californianus* in California [19]. As well as some of the cold-blooded organisms that have the capability of concentrating the *T. gondii* oocysts through the consumption of other marine mammals like the eastern oysters *Crassostrea virginica* and California mussels *Mytilus californianus*; *Toxoplasma gondii*is unable to infect those organisms, however, the oocysts can be concentrated in their digestive tract [24, 25]. The bioaccumulation of *T. gondii* oocyst in consumed oysters or mussels, which are often eaten raw, can transmit pathogens present in the water and directly indicate health risks to humans [26]. Bivalve mollusks, although not domestic animals, can act as carriers and hosts for *T. gonii* oocyst [27, 28]. These pathogens are stored in their tissues [20, 30]. The objective of the present work is detecting *Toxoplasma gondii* parasites in the *Helix pomatia* and identifying infected organs (such as the intestines, stomach, and pod).

Materials and Method

Sample collection

One hundred specimens of *Helix pomatia* snail were collected from a variety of agricultural fields in Wasit province - Iraq, between (February - March / 2020), and samples were taken from (stomach, intestine and pod) These samples were kept at a temperature of (-20°C), until they were used to extract DNA.

DNA Extraction

Genomic DNA has been obtained from the samples of the tissues through the use of the gSYNCTM DNA Extraction Kit (Geneaid. U.S.).

The probe and primers

Certain probe and primers targeted 94bp fragment amplification from B-1 gene of the *Toxoplasma gondii* in a variety of the samples. Those probe and primers have been designed by [31] and supplied by (Macrogen company, Korea), the sequences of primer TOXO-R (5'- AGCGTTCGTGGTCAACTATCGATTG - 3') and TOXO-F (5'- TCCCCTCTGCTGGCGAAAAGT - 3'); and the sequences of TaqMan probe which are specific for the B-1 gene in the *Toxoplasma gondii,* was (5-6 FAM-TCTGTGCAACTTTGGTGTATTCGCAG -TAMRA-3).

Real-Time PCR

This method has been conducted based on the method which has been presented in [31]. qPCR master mixture has been produced with the use of the (GoTaq®q-PCR Master mixture) which has been made following the instructions of the manufacturer, totally of 20µL of the solution of reaction (DNA template 5.0µL; B1 Forward primer (10pmol) 1.0µL; B1 Reverse primer (10pmol) 1.0µL; B1 probe (20pmol) 1.0µL; q-PCR master mixture10µL; PCR water 2µL) placed in the Real-time PCR Thermo-cycler (Bio-Rad . U.S.).

Results and Discussions

The snail enteric sample results in the real time PCR have been summarized in the figure1, there were 6 positive samples out of 32 enteric sample, the maximal concentration level of the DNA fragment of the B-1 gene of the *Toxoplasma gondii* amplifications of those samples has been (0.844) and the minimal has been (0.716), while there were 2 positive sample out of 32 gastric sample with maximum concentration of DNA fragment of B-1 gene (0.744), Shown in Figure2. The pods samples have been negative.

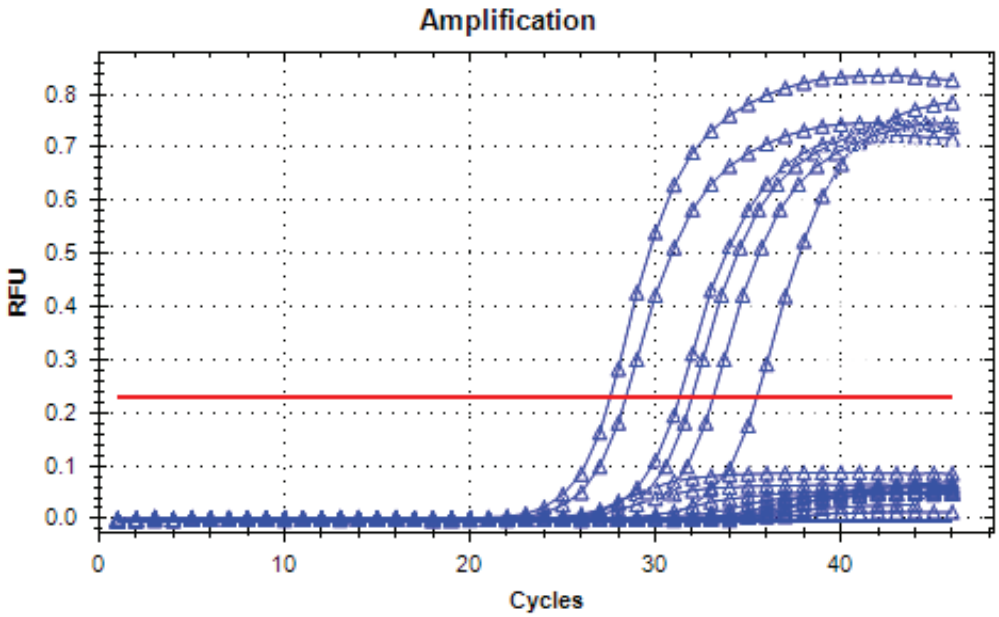

Fig.1: The plot of the Real-Time PCR amplification of the B-1 gene in the positive *Toxoplasma gondii* from the enteric snail samples

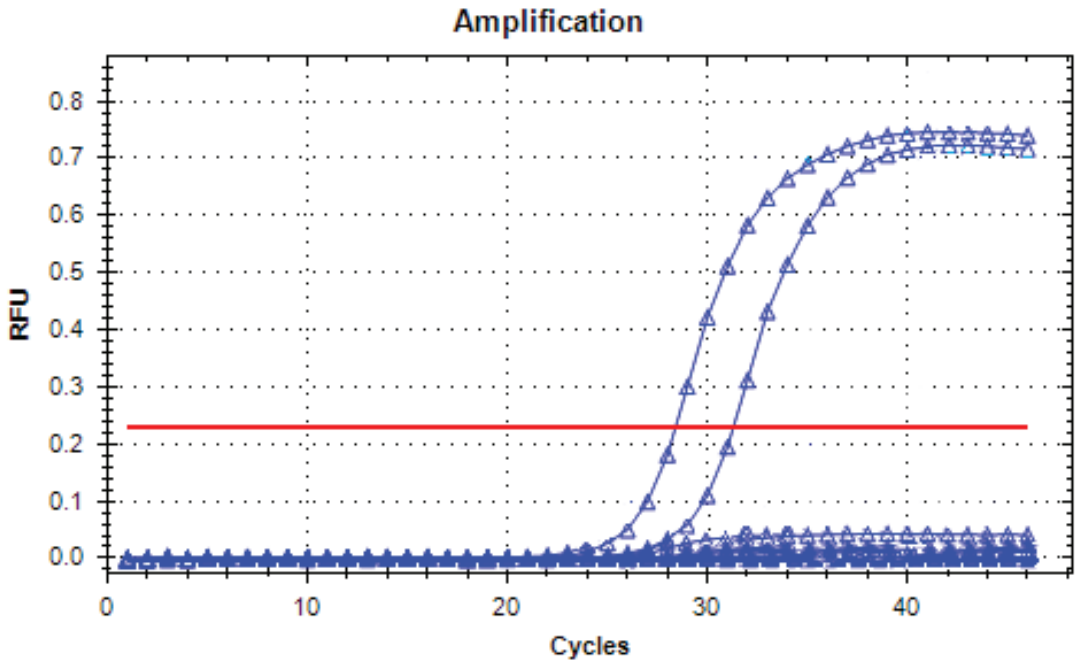

Fig.2: The plot of theReal-Time PCR amplification of the B-1 gene in the positive *Toxoplasma gondii* from the gastric snail samples

Earlier researches have shown that the parasites of the *Toxoplasma gondii* have been entirely terrestrial organisms, however, some of the authors' researches elucidated that *T. gondii* are infecting some of the marine animals like the sea otters as well as other types of the marine mammals [32, 33], which seldom consume the intermediate host containing parasites.

Cool *et al.*,(2000) [34] have shown that different types of invertebrates are vectors for the parasite, where the oocyst of the parasite are concentrated in some types of invertebrates such as bivalve oysters as a result of contamination of the aqueous medium, which leads

to transmission of infection to other organisms when consuming them as food [35]. However, in the present research, Iraqi rivers (Euphrates and Tigris) have a small number of the filter-feeding invertebrate species [36] Therefore, the likelihood to obtain positive samples was small.

Massie *et al.*, (2010) [25] have suggested that *Toxoplasma gondii* do not have the capability of infecting the filter-feeding invertebrates however, the oocysts can be concentrating within their organs like the digestive tracts as well as other organs which are in close contacts with the water that contains those organisms**.** The adverse result of pod samples may be due to the failure of filtration the infectious stages of parasite into the organs that close contact with the environment or the inability of the parasite to survive in these organs.

experimentally Arkush *et al.,* (2003))24) demonstrated that samples of mollusks of the type *Mytilus galloprovincialis* contained ssRNA of the *Toxoplasma gondii* parasite after using different concentrations of the *T.gondii* oocyst. and showing that the *T. gondii* oocyst stay infectious even after they pass through the marine invertebrate digestive system like in the oysters and mussels. On the other hand Coupe *et al.,* (2019) [37] used a variety of PCR-based methods to confirm the presence of the *Toxoplasma gondii* parasite›s DNA in the green mussel *Pern canaliculus* using 2 Nested-PCR assays that target the dhps gene and B1 gene and 2 Real time-PCR assays that target B1-gene and the repeating element 529-bp By comparing the results of the two assays, it was concluded that the rep529 qPCR test may be preferred for future mussel studies, but the DNA sequence is required for final confirmation of the detection of *T. gondii* DNA. However, there remain no evidences about the human infection by the *T. gondii* as a result of the consumption of the raw meat of the sea animals or their products.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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