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Molecular Detection of *Toxoplasma gondii* in Native Freshwater Fish *Cyprinuscarpio* in Wasit Province Iraq

Altamemy A. K. Aakool[1], Shiemaa Jabbar Abidali[2]

[1]Wasit University, College of Science, Department of Biology
[2]Directorate General of Education Wasit

Abstract: ***DNA of total 96 samples of (24) muscle, (24) liver, (24) enteric and (24) gills of native freshwater fish Cyprinuscarpio was extracted by Genomic DNA purification kit and tested using Real time polymerase chain reaction and specific set of primers and probe to detect B1 gene of Toxoplasma gondii. Sixteen enteric samples and only two gills samples was positive whereas the others was negative, this results refers to presence of T. gondii in this species of fish, a based to these results the infection may be acquired either from contaminated environment or due to consuming filter concentration invertebrates. The negative results of muscle and liver may be due to localization of parasites in organs that close contact with environment and the parasites either have no ability to diffuse to visceral organs or have no capable to survive in these organs.***

Keywords: Toxoplasma fish IRAQ real time PCR

1. Introduction

Toxoplasmosis is a zoonotic protozoon disease caused by protozoan parasite *Toxoplasma gondii*, it is an obligatory microscopic parasite considered one of most successful eukaryotic pathogen in the world it is widely prevalent in human, birds and other terrestrial animals (1, 2, 3). Toxoplasmosis is one of the one dangers disease because its implication in abortion and congenital disease in it intermediated hosts, and have very rang in infection from flu-like symptoms in immunocompetent adults to high disseminated disease in immunesuppressed individuals to birth defects infants when women are exposed during pregnancy .Toxoplasmosis infection results in generalized parasitemia involvement of brain, liver lung and other organs, and often death (1, 3, 4, 5). Oocyst survive ability of this parasite depending on environmental conditions, parasite can live in high and low humidity, hot and dry regions (6).Although the final hosts of *T. gondii* are terrestrial, a various species of marine mammals are among the documented intermediate hosts (7). In fact, The first explosion of *T. gondii* in a marine mammal was designated in 1973 by Van Pelt and Dietrich in a harbor seal (8). *Toxoplasma gondii* looks to be a strong constraint to southern sea otter population growth and a threat to the long-term survival of the subspecies (9) ; this was unexpected, as sea otters do not ingest the usual intermediate hosts of *T. gondii* and their location in sea water saves them isolated from cats litters, definitive proof that *T. gondii* was killing the sea otters came when viable *T. gondii* was isolated from the muscles of sea otters (10, 11) . It has been postulated that *T. gondii* oocysts defecated in the feces of feral cats living along the Pacific coast enter the marine (12). Tachyzoites of *T. gondii* were experiential to survive for up to seven days when cultured on goldfish cells at 37°C, but these results do not demonstrate that infection of poikilotherms is epidemiologically significant, or even occurs under natural conditions (13). Esmerini *et al.*, (2010) (14) and Miller *et al.*, (2008) (15) identified naturally acquired *T. gondii* using multi-locus PCR and DNA sequencing in mussel, and also by nested PCR targeting B1gene locus in 3.3% of a 300 oysters samples.

The capacity of bivalves to acquire pathogens from sea water is well known (14, 15, 16, 17). The bivalves are severely preyed upon by sea otters in some areas of the California coast; acquisition and concentration of *T. gondii* oocysts by bivalves seems likely to play a potentially important role in the mechanism of *T. gondii* transmission from terrestrial felids to sea otters (15).
The sea otters that preyed upon marine snails had an 11-fold increased risk of *T. gondii* infection, whereas predation upon bivalves was not a significant risk factor (18). *Toxoplasma gondii* infect several marine mammal species, such as seals, blowfish, dolphins, porpoises, whales, sea lions and sea otters (15, 19, 20, 21, 22). Under natural conditions, *T. gondii* was recently detected in wild shellfish such as *Mytiluscalifornianus* in California (15), and in *Mytellaguyanensis* and *Crassostrearhizophorae* in Brazil (14). Some cold-blooded organisms, which are able to concentrate *T. gondii* oocysts by consumed other marine mammal such as California mussels *Mytiluscalifornianus* and eastern oysters *Crassostreavirginica* and migratory fish such as Sardines *Sardinopssagax*and anchovy *Engraulismordax; Toxoplasma gondii*is not capable of infecting these organisms but oocysts may be concentrated in their digestive tracts (23, 24).

Studied showed that sero-positivity to *T. gondii* is relatively high (60%) among Inuit communities in Nunavik, Canada, One of the significant risk factors was consumption of raw seal, walrus or white dolphin meat (18).

Previous studies have shown accumulation of *T. gondii* in zebra mussel is due to exposure to low oocysts concentrations (25).

The aim of this study detection the *toxoplasma gondii* parasites in native *Cyprinuscarpio* fish and identified the infected organs (muscle, liver, intestine and gill).

2. Materials and Method

Sample collection

A twenty four samples of widespread freshwater fish *Cyprinuscarpio* collected with 2.5 to 3 kg weight from different location of Tigris river and its branches in Wasit province - Iraq, tissue samples were collected from muscle, liver, intestinal and gills which was grind using liquid nitrogen and grinder to prepare tissue powder.

DNA purification

The DNA of samples was extracted by Genomic DNA purification kit (promega. USA).

The primers and probe

A specific primers and probe targeted the amplification of 94 bp fragment from the B1 gene of *Toxoplasma gondii* in different samples. These primers and probe were design by (26) and provided by (Bioneer company, Korea), the sequences of primerTOXO-F (5'-TCCCCTCTGCTGGCGAAAAGT-3') and TOXO-R (5'-AGCGTTCGTGGTCAACTATCGATTG-3'); the TaqMan probe specific for B1 gene in *Toxoplasma gondii*, the sequences of this probe was (5-6FAM-TCTGTGCAACTTTGGTGTATTCGCAG -TAMRA-3).

Real-Time PCR

This technique was carried out according to method described by (26). The qPCR master mixwas prepared by using (**GoTaq®qPCR Master mix**) and this master mix done according to company instructions, a total of 25 µL volume of reaction solution (DNA template 5µL; B1 Forward primer (10pmol) 1µL; B1 Reverse primer (10pmol) 1µL; B1 probe (20pmol) 1µL; qPCR master mix 12.5 µL; PCR water 4.5 µL) placed in Real-time PCR Thermocycler (BioRad . USA).

3. Results and Discussion

The results of fish enteric samples in real time PCR are summarized in figure (1), there are 16 out of 24 enteric samples was positive, the maximum concentration of DNA fragment of B1 gene of *Toxoplasma gondii* amplification of these samples was (1.16) and the minimum was (0.487), whereas two out of 24 samples of fish gills was positive with endpoint analysis of B1 gene (0.744), figure (2). The other sample of muscle and liver was negative.

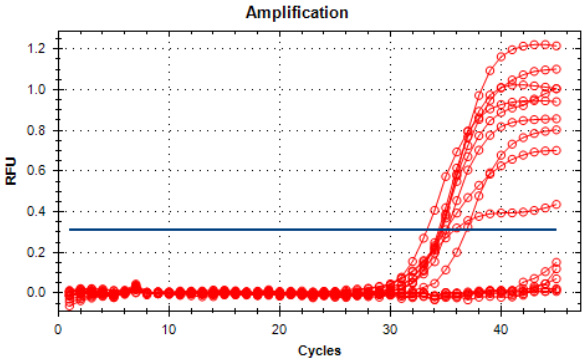

Figure 1: Real-Time PCR amplification plot of B1 gene in positive *Toxoplasma gondii* from fish enteric samples

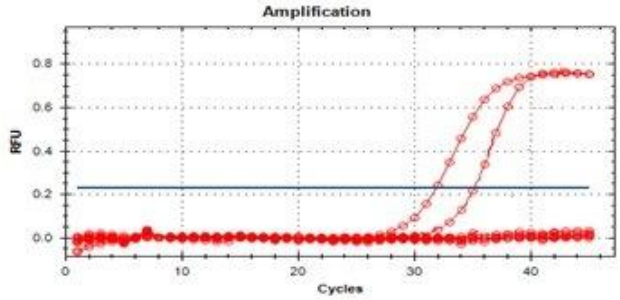

Figure 2: Real-Time PCR amplification plot of B1 gene in positive *Toxoplasma gondii* from fish gill samples

The previous studies refers that the *Toxoplasma gondii* parasites was exclusively terrestrial organisms, but some authors investigations elucidate that the *T. gondii* infect some marine animals such as sea otters and other marine mammals (27, 28), these animals rarely consume intermediate host containing parasite. Cole *et al*., (2000) (10) suggested that the infection of marine mammals due to ingesting infected invertebrates which could is phoretic agents for *T. gondii* oocysts. Oocysts would contaminate the marine environment through storm runoff (29) or sewage (20) and would be concentrated by some (filter-feeding) invertebrates, like bivalve shellfish, consequently which serving as a source of infection when consumed by for marine animals as nutrient materials (11, 23). But in this study the Iraqi rivers (Tigris and Euphrates) have a few filter-feeding invertebrates species (30**)**, so the chance of infection of native river fish (*Cyprinuscarpio)* through contamination of the rivers with storm runoff or sewage that draining directly to these rivers. Massie *et al*., (2010) (24) suggestion that the *Toxoplasma gondii* haven't ability to infect filter-feeding invertebrates but oocysts may be concentrated in their organs such as digestive tract and other that close contact with water containing these organisms.The negative results of muscle and liver in our study may be due to localization of parasites in organs that close contact with environment and the parasites either have no ability to diffuse to visceral organs or have no capable to survive in these organs. Experimentally the tachyzoites of *T. gondii* were observed to survive more than seven days goldfish culture cells at 37°C, but this not indicates that the infection of cold blood organism epidemiologically significant under natural conditions (13). On the other hand (31) refers to presence specific immunoglobulin M (IgM) and immunoglobulin G (IgG) of *T. gondii* serum samples of Salmonidae by using enzyme-linked immunosorbent assay (ELISA), according this findings they concluded that Salmonidae may be susceptible to *T. gondii* infection but there is still no evidence of *T. gondii* human infection due to consuming raw sea animal meat or their products.

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