Molecular Detection of *Toxoplasma gondii* in Different Type of Chicken’s Eggs in Iraq

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**Abstract**

This study aimed to detection *Toxoplasma gondii* in eggs of different sources, using Real- Time Polymerase Chain Reaction (PCR). A total of 300 chicken’s eggs samples were collected from different source (native chickens 100, Iraqi eggs production fields 100, trade-imported eggs 100). Genomic DNA extracted from inner components of eggs (chalaza) and tested by Real Time PCR using specific primers and TaqMan probe targeting B1 gene of parasite. The native eggs samples showed 64 of 100 (64%), 8 of 100 (8%) of Iraqi eggs production fields and 4 of 100 (4%) of imported eggs were detected as positive, the others samples was negative. Statistical analysis showed highly significant differences between the different sources when using Real - time (PCR) technique to detection *Toxoplasma gondii* under p ≤ 0.05. These results is indicate that *Toxoplasma gondii* infection in native eggs is relatively high in compare with other eggs sources.

**Keywords:** *Toxoplasma gondii*, chalaza, egg, chicken, Real -Time PCR.

**INTRODUCTION**

*Toxoplasma gondii* is an obligate intracellular protozoan parasite belongs to the Apicomplexa phylum that was worldwide distribution and capable infecting virtually all warm-blooded animal, including birds, humans, and other mammals [1, 2]. Human can infect by ingestion of oocysts shedding from definitive host, which contaminate the environment (soil, water and food) [3] or by tissue cysts in raw or undercooked meat of the intermediate hosts [4-6]. *T. gondii* can be also transmitted by a vertical transmission (via placenta) to unborn offspring were the modes of transmission of *T. gondii* first discovered in human and later found in other species of animals especially sheep, goats, and rodents [1]. Toxoplasmosis may lead to congenital defects with abortion during pregnancy [8, 9] or damages in eye and central nervous system [10, 11].

Birds, including Chickens, especially if free-range, play an important role in the epidemiology of *T. gondii* because their feeding style, therefore it is one of the good indicators for environmental contamination with *T. gondii* oocysts [12]. Chickens are an important source of infection for cats, while chicken meat as a source of infection for humans is less than other meats [13]. Domestic chicken show high infection rates with toxoplasmosis in all countries [14]. In experimentally toxoplasmosis in chickens, only one of 323 eggs had viable *T. gondii*; only one of the five mice inoculated with this egg homogenate became infected, this probably due to had a few organisms [15]. Another study, all 2214 eggs laid by experimentally infected hens was negative for *T. gondii* [15]. In both of these two experiments, all chickens had been inoculated intraperitoneal with large doses of *T. gondii* tachyzoites or tissue cysts. Congenital transmission in chickens unclear, however, one study showed that eggs contain very low percentage of *T. gondii* parasite [16]. While other study showed a substantial embryonic mortality and malformation of surviving chicks (18%) following experimental toxoplasmosis in hens [17].

**MATERIALS AND METHODS**

**Sampling**

A total of 300 hens’ eggs were collected randomly from different source, 100 from Iraqi eggs production fields, 100 from native chickens and 100 trade-imported from different sources, chalaza was obtained with a little amounts from other inner components to DNA extraction.

**Genomic DNA Extraction**

DNA was extracted using Genomic DNA Mini kit (Geneaid, USA) in accordance to the manufacturer’s instruction. The samples was preserved at -20’c to use later.
Real-Time Polymerase Chain Reaction

The Real-time PCR amplification designed with the primer express software (PE Applied Biosystem) to specifically amplify the T. gondii B1 gene. The target DNA for real-time PCR amplification was the published sequence of the 10-fold repetitive B1 gene of the T. gondii and the 94bp repeat element sequence, and Taq-Man probe to DNA amplification using Taq-Man technology and at the 7500 fast real-time PCR System (Applied Biosystem). The reaction solution was prepared at final volume 20μl containing, 10 μl2 of qPCR master mix (Genes Laboratories; USA), 1μl of forward primer TOXO-F (5'-TCCCCTCTGCTGCGAAGT-3'), 1μl of reverse primer TOXO-R (5'-AGCGTTCTGCTGCAACTATCGATTG-3'), 2 μl PCR mastermix, 5μl DNA template and 1μl of Taq Man probe (6FAM-TCTGTGCAACCTTTGGTGTT ATTCGCAG-TAMRA).

Cycling conditions were as follows: initial denaturation at 95°C for 5 min, 45 PCR cycles of denaturation at 95°C for 20 s and Annealing / Extension at 60°C for 30 s were performed. The cycle threshold value (C_T), indicative of the quantity of target gene at which the fluorescence exceeds a preset threshold, was determined. This threshold defined as 20 times the standard deviation of the baseline fluorescent signal, i.e., the normalized fluorescent signal of the first few PCR cycles. The positive samples exceeding threshold.

Statistical analysis

The Chi square test was used to evaluate significant differences (P ≤ 0.05) of infection rate in eggs of different sources of the sample collection.

RESULTS AND DISCUSSION

The results of Real-Time PCR preferred in table 1 that elucidate detection of Toxoplasma gondii B1 gene in some fence’s eggs, there is 64 of 100 (64%) of native eggs infected with toxoplasmosis (fig.1), 8 of 100 (8%) Iraqi eggs production fields (fig.2), and 4 from 100 (4%) of imported eggs were detected as positive samples, the others samples was negative (fig.3). This results was significantly (P≤0.05) high in native eggs compared to %) Iraqi eggs production fields and imported eggs.

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Total examined</th>
<th>Positive</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native eggs</td>
<td>100</td>
<td>64</td>
<td>64%</td>
</tr>
<tr>
<td>Iraqi eggs production fields</td>
<td>100</td>
<td>8</td>
<td>8%</td>
</tr>
<tr>
<td>Imported eggs</td>
<td>100</td>
<td>4</td>
<td>4%</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>76</td>
<td>25.3%</td>
</tr>
<tr>
<td>Chi-Square (χ²) **(P&lt;0.01)</td>
<td>--</td>
<td>--</td>
<td>12.794%**</td>
</tr>
</tbody>
</table>

**Fig-1:** The amplification cycles of T. gondii B1 gene fragment in native eggs samples by Real-Time PCR
Although Peixoto and Lopes [18] obtained *T. gondii* from ovaries and oviducts of naturally infected hens, the eggs shelled were not to be found infected with parasites [15], this results disagreement with our investigation in which detection of infection in eggs shelled of all samples. Elevation of infection in native eggs due to divergently nutrient sources where the chickens free living without cages or limited field where high distribution of parasites infective stages [19] in addition to extend of management period in houses more than other types of chickens. But the levels of infection in other eggs that obtained from Iraqi fields or imported from other countries where the chickens alive under critical hygiene management that decreased exposure to different infective stages of parasites with exception of presence rodents a live in feed storages that considered important sources of infection [9]. Humans should not be consumed raw hen eggs, for fear of acquiring infective stages of *T. gondii*, raw hen eggs are unlikely to be a source of infection for humans.

### CONCLUSION

The Real Time PCR technique was a very sensitive technique for diagnosis of DNA of *Toxoplasma gondii*, therfor we used in this study to detect parasite in inner component of raw eggs (chalaza) from different sources. The current study has demonstrated the presence of *Toxoplasma gondii* in different types of eggs but was a difference in the incidence it was (64%) in native eggs and (8%) in Iraqi eggs production fields while in imported eggs it was (4%). Statistical analysis showed highly significant differences between the different sources when using Real-time (PCR) technique to detection *Toxoplasma gondii* under p ≤ 0.05. These results is indicate that *Toxoplasma gondii* infaction in native eggs is relatively high in compare with other eggs sources.

### Acknowledgment

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