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

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Abstract | **Original Research Article**

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 fields100, 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 detec*ion Toxoplasma gondii* under $p \leq 0.05$.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 the10 - fold repetitive B1 gene of the T. gondii and the 94bp repeat element sequence, and TaqMan probe to DNA amplification using Taq-Man technology and at the7500 fast real-time PCR System (Applied Biosystem). The reaction solution was prepared at final volum 20µl containing, 10 µl2× of qPCR master mix (Genes Laboratories; USA), 1µl of forward primer TOXO-F (5′-TCCCCTCTGCTGGCGAAAAGT-3′), 1µl of reverse primer TOXO-R (5′-AGCGTTCGTGGTCAACTATCGATTG-3′), 2 µl PCR mastermix, 5µl DNA template and 1µl of Taq Man probe (6FAM-TCTGTGCAACTTTGGTGT 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 ($P \leq 0.05$) 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 ($P \leq 0.05$) high in native eggs compared to %) Iraqi eggs production fields and imported eggs**.**

Table-1: A total number of sample and positive Real-Time PCR results.

Type of sample	Total examined	Positive	Percentage %
Native eggs	100	64	64%
Iraqi eggs production fields	100	8	8%
Imported eggs	100	4	4%
Total	300	76	25.3%
Chi-Square (χ^2)	--	--	12.794%**

**(P<0.01)

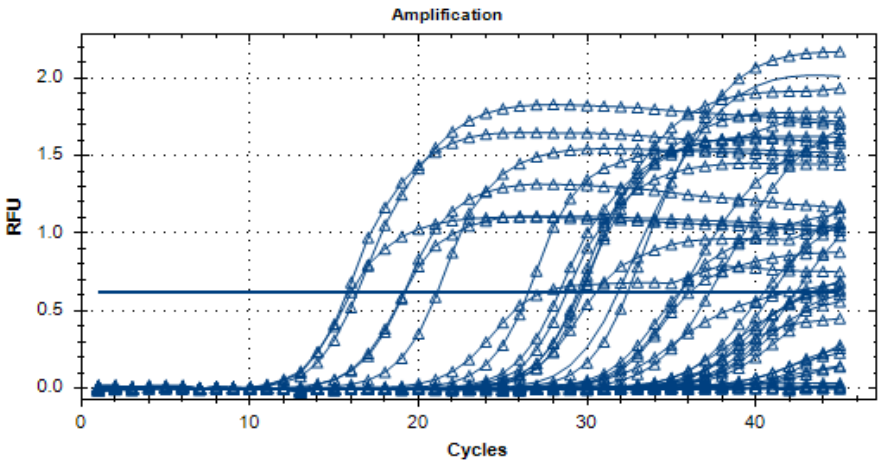

Fig-1: The amplification cycles of *T. gondii* B1 gene fragment in native eggs samples by Real-Time PCR

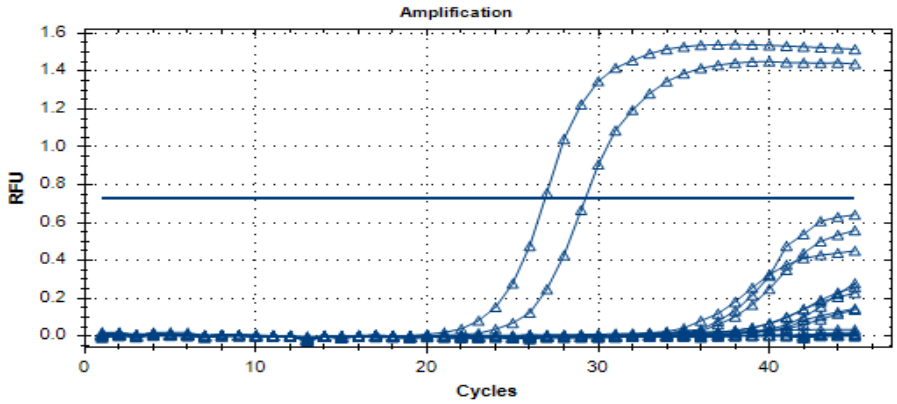

Fig.2 The amplification cycles of *T. gondii* B1 gene fragment in Iraqi fields eggs samples by Real-Time PCR

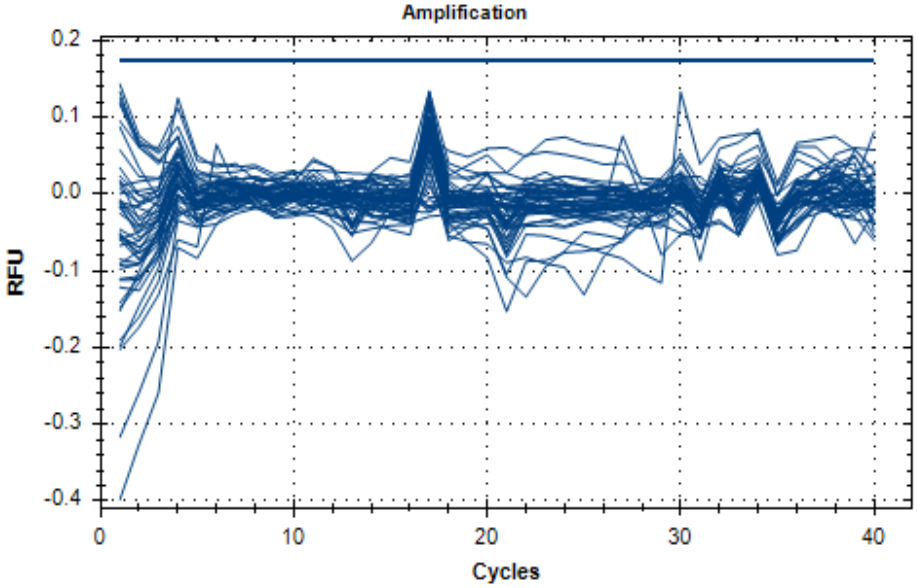

Fig-3: The negative eggs samples amplification cycles of *T. gondii* B1 gene fragment by Real-Time PCR

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 detect*ion Toxoplasma gondii* under $p \leq 0.05$.These results is indicate that *Toxoplasma gondii* infaction in native eggs is relatively high in compare with other eggs sources.

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REFERENCES

1. Robert-Gangneux F, Dardé ML. Epidemiology of and diagnostic strategies for Toxoplasmosis. Clin. Microbiol. Rev. 2012; 25 (2): 264-296.
2. Flegr J. Influence of latent Toxoplasma infection on human personality, physiology and morphology: pros and cons of the Toxoplasma–human model in studying the manipulation hypothesis. Journal of experimental Biology. 2013 Jan 1;216(1):127-33.
3. Lilly EL, Wortham CD. High prevalence of Toxoplasma gondii oocyst shedding in stray and pet cats (Felis catus) in Virginia, United States. Parasites & vectors. 2013 Dec;6(1):266.
4. Jones JL. DubeyJP. Foodborne toxoplasmosis. Clin. Infect. Dis. 2012; 55 (6), 845- 851
5. Juránková J, Basso W, Neumayerová H, Frencová, A, Baláž V, Deplazes P, Koudela B. Predilection sites for Toxoplasma gondii in she tissue serevealed by magnetic captur and real –time PCR detection. Food Microbiol. 2015; 52:150-3.
6. Hill DE, Dubey JP. Toxoplasma gondii. InFoodborne Parasites. 2018:119-138. Springer, Cham.
7. Dubey JP. Toxoplasmosis of Animals and Humans. CRC Press, Boca Raton, FL, USA. 2010a; 313.
8. Halsby K., Guy E, Said B, Francis, J. (Enhanced surveillance for toxoplasmosis in and Wales, 2008-2012. Epidemiol Infect. 2014; 142:1653–60.
9. Alvarado-Esquivel C, Vázquez-Alaníz F, Sandoval-Carrillo AA, Salas-Pacheco JM, Hernández-Tinoco J, Sánchez-Anguiano LF, Liesenfeld O. Lack of association between Toxoplasma gondii infection and hypertensive disorders in pregnancy: a case–control study in a Northern Mexican population. Parasites & vectors. 2014 Dec;7(1):167.
10. Moncada PA, Montoya JG. Toxoplasmosis in the fetus and newborn: A update on prevalence, diagnosis and treatment. Expert Rev. Anti-Infect. Ther. 2012; 10 (7): 815-828.
11. Jeong WK, Joo BE, Seo JH, Mun JK, Kim J, Seo DW. Mesial temporal lobe epilepsy in congenital toxoplasmosis: a case report. J Epilepsy Res. 2015; 5:25–8.
12. Dubey JP. Toxoplasma gondii infections in chickens (Gallus domesticus): prevalence, Clinical disease, diagnosis, and public health significance. Zoonoses and Public Health. 2010b; 57: 60–73.
13. Dubey JP, Lehmann T, Lautner F, Kwok, OCH, Gamble, HR. Toxoplasmosis in sentinel chickens (Gallus domesticus) in New England farms: seroconversion, distribution of tissue cysts in brain, heart, and skeletal muscle by bioassay in mice and cats. Vet Parasitol. 2015;214:55–58.
14. Dubey JP, Graham DH, Blackston CR, Lehmann T, Gennari SM, Ragozo AM, Nishi SM, Shen SK, Kwok OC, Hill DE, Thulliez P. Biological and genetic characterisation of Toxoplasma gondii isolates from chickens (*Gallus domesticus*) from Sao Paulo, Brazil: unexpected findings. International journal for parasitology. 2002 Jan 1; 32(1):99-105.
15. Jacobs L, Melton ML. Toxoplasmosis in chickens. J. Parasitol. 1966; 52: 1158–1162.
16. Iannuzzi L, Renieri T. The egg in the epidemiology of toxoplasmosis: tests of experimental infections by injection through the shell. Acta. Med. Vet. 197;117: 311- 317.
17. Caballero-Servin A. Congenital malformations in Gallus gallus induced by Toxoplasma gondii. Rev Invest Salud Publica (Mexico). 1974; 54:87–94.
18. Peixoto CM, Lopes CW. Isolamento do Toxoplasma gondii Nicolle & Manceaux, 1909 (Apicomplexa: Toxoplasmatinae) em galinhas naturalmente infectadas. Arq. Univ. Fed. Rur. J. 1990;13:99-103.
19. Liu XC, He Y, Han DG, Zhang ZC, Li K, Wang S, Xu LX, Yan RF, Li XR. Detection of Toxoplasma gondii in chicken and soil of chicken farms in Nanjing region, China. Infect Dis Poverty. 2017; 6: 62.