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Molecular Detection of *Toxoplasma gondii* in Chicken Meats and Eggs in Semnan City, Iran

Fatemeh Mehrabi[1], Maryam Rassouli [1,2*], Seyed Hesamodin Emadi Chashmi[3]

[1] Pathobiology Department of Veterinary Faculty, Semnan University, Semnan, Iran

[2] Pathobiology Department of Shahmirzad School of Veterinary Medicine, Semnan University, Semnan, Iran

[3] Department of Clinical Sciences of Veterinary Faculty, Semnan University, Semnan, Iran

Abstract

Background: *Toxoplasma gondii* is a protozoan parasite, phylum Apicomplexa. Felids are definitive hosts and all warm-blooded animals and humans are intermediate hosts. The clinical symptoms of toxoplasmosis among chickens are mostly subclinical but the

infection of chickens and eggs is important as a source of protein for human consumption.

Objectives: The study aimed to detect *T. gondii* in chicken meat and egg by molecular examination.

Methods: In this study, 100 chicken legs, 50 eggs of free-range hens, and 50 eggs of industrial hens were collected from different stores in Semnan city, Iran. The samples were inspected for the *Toxoplasma* B1 gene after DNA extraction.

Results: According to the results, *Toxoplasma* DNA was detected in 23% of chicken legs, 36% of eggs of free-range chickens, and 20% of eggs of industrial chickens. The infection rate was not significantly different between eggs of free-range and industrial chickens ($P>0.05$).

Conclusion: Therefore, *Toxoplasma* is present in chicken meats and eggs in Semnan city, Iran, and it is recommended that people eat well-cooked chicken meat and eggs for disease control and also feed the domestic carnivores with cooked meat to prevent the parasite life cycle.

Keywords: Avian, *Gallus gallus*, Poultry, Prevalence, Toxoplasmosis

Introduction

Toxoplasma gondii is an obligatory intracellular zoonotic protozoan parasite that belongs to the cyst forming coccidia group in the phylum Apicomplexa. Felids are definitive hosts and the sexual phase is passed in their intestines. Oocysts are excreted through feces in the environment, and then become sporulated and infective. It means the oocyst contains two sporocysts and each sporocyst has four sporozoites (Gajadhar, 2015).

All warm-blooded animals and humans can be intermediate hosts. The asexual phase is passed in the intermediate host body and tachyzoites and bradyzoites (tissue cysts) are formed (Dubey, 2021).

Birds can be infected *T. gondii* by ingestion of infective oocysts that are shed from felids in soil or water. Sporozoites enter the cells and rapidly replicate as tachyzoites, the host immune system is activated and the parasite replication becomes slow and tachyzoites change to bradyzoites confined in tissue cysts (Gajadhar *et al.,* 2006; Dubey, 2010). Cysts can be formed in the brain, spinal cord, eye, lymph nodes, heart, liver, lung, kidney, and muscles. Contamination of felids and other carnivores occurs by ingestion of these infected organs in prey-predator relations. Birds are also a source of protein for humans and if they are consumed undercooked, people can be also infected (Dubey, 2010).

Toxoplasma gondii also has a vertical transmission and can infect eggs before laying. If the eggs are consumed raw, they can be the source of infection for carnivores and humans (Gajadhar, 2015; Chumpolbanchorn *et al.,* 2013).

Free-range chickens (*Gallus domesticus*) have the most potential host for ingestion of *T. gondii* oocysts from the soil. They are also used for epidemiological studies to investigate the soil contamination of *T. gondii* but they rarely show clinical symptoms (Dubey *et al.,* 1993; Kaneto *et al.,* 1997; Dubey, 2010).

Toxoplasma gondii can be diagnosed by different kinds of techniques such as tissue smears, dye test, serology, histopathology, immunohistochemistry, bioassay, and molecular examinations (Sabin and Feldman, 1948; Munday and Carbould, 1971;

Remington *et al.,* 2011; Ortega-Mora *et al.,* 2007; Burg *et al.,* 1989; Gutierrez *et al.,* 2010).

This study aimed to detect *T. gondii* DNA (deoxyribonucleic acid) in chicken meats and eggs as two main sources of protein for human in Semnan city, Iran.

Materials and Methods

Sample collection

One hundred fresh chicken legs were purchased at different stores in Semnan city, Iran. The legs were packed separately, labeled, and then transferred immediately to a 4˚C refrigerator. 100 mg of each leg was cut by a sterile scalpel and transferred to a sterile microtube and stored in a -20˚C freezer for DNA extraction.

Fifty eggs of free-range chickens and fifty eggs of industrial chickens were also purchased from different stores in Semnan city, Iran. Eggs were broken into 50 ml sterile tubes then mixed well, labeled, and stored in a -20 ˚C freezer for DNA extraction.

DNA extraction and molecular examination

For chicken meat DNA extraction about 100 mg of tissue was homogenized by mortar in a sterile tube. 1ml of homogenized eggs were also transferred to 2 ml sterile tubes. Tris-HCl (pH 8·0) and proteinase K (Fermentas®, Lithuania) (200 µg/ml) were added to the samples. The samples were incubated at 55 ˚C for 2h. The DNA extraction method was based on the phenol-chloroform and ethanol precipitation method. The purified DNA samples were stored in 50 ml of TE buffer (10mM Tris and 1 mM EDTA, pH 8·0 at −20 °C.

Extracted DNA of the *Toxoplasma* RH strain (kindly provided by Medical School of Zanjan University of Medical Sciences & Health Services, Zanjan, Iran) was used as a

source of a positive control sample. Sterile distilled water instead of DNA was used as a negative control.

B1 gene of *Toxoplasma* (35 copies per parasite) was amplified by using Nested PCR with 2 sets of oligonucleotide primers; Forward primer1: 5′-GGAACTGCATCCGTTCATGAG-3′, Reverse primer1 5′-TCTTTAAAGCGTTCGTGGTC-3′, Forward primer2: 5′-TGCATAGGTTGCAGTCACTG-3′, and Reverse primer2: 5′-GGCGACCAATCTGCGAATACACC-3′ (Burg *et al.*, 1989). Amplification was conducted in 20 µl reaction volumes (ParsTous PCR premix kit, Iran). 10 pmol of each PCR primer (Takapouzist Co. Iran), and 1µl of DNA template (250–500 ng) were added to each reaction and the remaining 20 µl reaction volume was filled with sterile distilled water. The reactions were subjected to the following cycling conditions in Bioer thermocycler: 94 °C for 3 min, 40 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min, followed by a final extension at 72 °C for 7 min. The first PCR products were diluted at 1:10 then the second round of PCR was performed on all the first PCR products (with 193bp band and without). The annealing temperature of the Nested-PCR was 52 °C and the number of cycles was 30, the other temperatures were the same as the first PCR. For observation of 96bp bands, the Nested-PCR products were stained by ethidium bromide and electrophoresed through a 1.5% agarose gel. For amplification size evaluation, a 100 bp plus molecular marker (Sinaclone ®, Iran) was used.

Statistical analysis

The infection rate between two kinds of eggs (eggs of free-range and industrial chickens) was analyzed by Chi-square test, SPSS software. $P<0.05$ was considered a significant difference between the two groups.

Results

Toxoplasma gondii DNA was detected in 23 out of 100 chicken legs (23%, 14.8-31.2% with a 95% confidence interval (CI)), 18 out of 50 eggs from free-range chickens (36%, 22.7-49.3% with a 95% CI), and 10 out of 50 eggs from industrial chickens (20%, 9-31% with a 95% CI) (Figure 1.). In the Chi-square test, the X^2 value in infection rate between eggs of free-range and industrial chickens was 3.1746 and there was no significant difference between these groups ($P>0.05$).

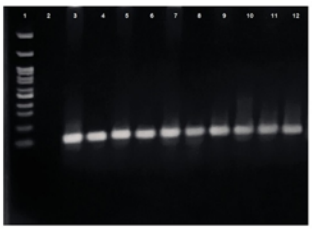

Figure 1. PCR products on agarose gel, lane 1: 100 bp plus molecular marker, lane 2: negative control, lane 3: positive control, lanes 4-12: 96bp bands of *Toxoplasma gondii* in some samples.

Discussion

After observation of relatively high *Toxoplasma* seroprevalence among free-range chicken (96.7%) and industrial chickens (39.9%) in Semnan city, Iran by ELISA

(enzyme-linked immunosorbent assay) technique (Hosseini *et al.,* 2019), *Toxoplasma* DNA was detected in 23 out of 100 chicken legs (23%) in this study.

Toxoplasma gondii DNA has been detected in chicken meat in different studies; In Iran, *T. gondii* DNA has been detected in 8% out of 50 chicken meat samples (Mahami-Oskouei *et al.,* 2017). In Canada, 3.9% out of 234 samples (Iqbal *et al.,* 2018), In Argentina, 30.3% out of 33 samples (Bernstein *et al.,* 2018), In Brazil 40% out of 40 samples (Holsback *et al.,* 2012), and 16.7% out of 12 (Fernandes *et al.,* 2016), In Caribbean Islands 28% out of 81 samples (Hamilton *et al.,* 2017), and 19.1% out of 162 samples (Hamilton *et al.,* 2019), In China 8.2% out of 257 samples (Zou *et al.,* 2017), 12.3% out of 1653 samples (Sun, 2018), and 2.2% out of 360 potions of meat of industrial chickens and 19.2% out of 360 portions of meat of free-range chickens (Wang *et al.,* 2020), In Colombia 35% out of 40 samples (Campo-Portacio *et al.,* 2014), In Kenia 79% out of 105 samples (Mose *et al.,* 2016, 2017), In Pakistan, 20% out of 65 meat samples of free-range chickens and 10.8% out of 230 meat samples of industrial chickens (Khan *et al.,* 2020) had *T.gondii* DNA in molecular examinations.

In the US, no viable *T.gondii* has been detected in chicken breasts out of 2095 samples which may be a cause of the low detection possibility of *T. gondii* in chicken breasts (about 18%) (Dubey *et al.,* 2005).

Toxoplasma gondii has been detected in the hen ovaries and oviducts (Jacobs and Melton, 1966; Dubey, 2021). In some experimental studies, *Toxoplasma* infection of eggs was low, for example, 1 egg out of 322 eggs from infected hens had live *Toxoplasma* (Jacobs and Melton, 1966), live *T.gondii* was isolated from 6 out of 408 eggs from 22 infected hens (Pak, 1969) therefore, it concluded the vertical transmission rate of *T.gondii*

to chicken eggs was relatively low (Dubey, 2010) but by using molecular examinations as sensitive tests especially nested-PCR on B1 gene (Mason *et al.*, 2010) the infection rate of *T.gondii* in eggs has been reported increasingly. In Iraq, *T.gondii* DNA has been detected in 20% out of 30 eggs from free-range chickens (Al-Khanaq *et al.*, 2018), and in Iran, this number was 11% out of 200 eggs (Khademi *et al.*, 2018). In this study, *T. gondii* DNA was detected more in eggs in comparison with a similar study (Khademi *et al.*, 2018).

Toxoplasma is easily killed by cooking. The internal temperature of meat should reach 67 ˚C (Ito *et al.*, 1975) to kill *Toxoplasma* or the meat is frozen for 15 days at -20 ˚C. *Toxoplasma* in eggs is killed by boiling or frying (Gajadhar, 2015; Dubey, 2021).

Unfortunately, In Iran raw eggs and also undercooked chicken meat in Kebab are consumed by humans that both of them have potential risks for *Toxoplasma* transmission. This habit can be so dangerous for pregnant women and immunosuppressive individuals. It is also recommended to feed domestic carnivores with well-cooked food.

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Conflict of interests

The authors declare that they have no conflict of interests.

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ردیابی مولکولی توکسوپلاسما گوندی در گوشت مرغ و تخم مرغ در شهر سمنان، ایران

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فاطمه مهرابی[1]، مریم رسولی[2،1*]، سید حسام الدین عمادی چاشمی[3]

[1] گروه پاتوبیولوژی دانشکده دامپزشکی، دانشگاه سمنان، سمنان، ایران

[2] گروه پاتوبیولوژی آموزشکده دامپزشکی شهمیرزاد، دانشگاه سمنان، سمنان، ایران

[3] گروه علوم درمانگاهی دانشکده دامپزشکی، دانشگاه سمنان، سمنان، ایران

305 *ایمیل نویسنده مسوول: Mrvpar@semnan.ac.ir

خلاصه فارسی:

مقدمه: توکسوپلاسما گوندی تک یاخته اجباری داخل سلولی در شاخه اپی کمپلکسا می باشد. گربه سانان میزبان نهایی و تمامی حیوانات خونگرم و انسان میزبان های واسط توکسوپلاسما محسوب می شوند. آلودگی مرغ و تخم مرغ به این انگل عنوان منابع تامین پروتئین انسانی می تواند حائز اهمیت باشد.

هدف: ردیابی مولکولی توکسوپلاسما در گوشت مرغ و تخم مرغ به عنوان دو منبع مهم تامین پروتئین در انسان و حیوانات گوشتخوار می باشد.

روش کار: در این مطالعه 100 نمونه ران مرغ گوشتی جمع آوری شده از فروشگاه های سمنان و 50 تخم مرغ بومی و 50 تخم مرغ صنعتی جمع آوری گردید. نمونه ها پس از استخراج DNA مورد ردیابی ژن B1 توکسوپلاسما قرار گرفتند.

نتایج: بر اساس نتایج بدست آمده DNA توکسوپلاسما در 23٪ (14/8 - 31/2٪ با فاصله اطمینان 95٪) نمونه های عضله ران، 36٪ (22/7- 49/3٪ با فاصله اطمینان 95٪) تخم مرغ های بومی و 20٪ (9-31 ٪ با فاصله اطمینان 95٪) تخم مرغ های صنعتی ردیابی گردید، میزان آلودگی تخم مرغ های بومی و صنعتی اختلاف معنی داری نداشت ($P>0/05$).

نتیجه گیری: بنابراین مشخص گردید که انگل توکسوپلاسما در مرغ ها و تخم مرغ های عرضه شده در فروشگاه های سمنان حضور دارد. برای کنترل و پیشگیری از ابتلاء افراد به توکسوپلاسموز باید مرغ و تخم مرغ کاملا پخته شوند و برای جلوگیری از تکمیل چرخه توکسوپلاسما به گربه های خانگی حتما گوشت پخته خورانده شود.

کلمات کلیدی: پرندگان، توکسوپلاسموز، *Gallus gallus*، شیوع، ماکیان