Research Paper

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

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ABSTRACT

Background: Toxoplasma gondii is a protozoan parasite, a member of the 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: This 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 hens, and 20% of eggs of industrial hens. The infection rate was not significantly different between eggs of free-range and industrial hens (P>0.05).

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

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

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*** Corresponding Author:**
Maryam Rassouli
Address: *Pathobiology Department of Veterinary Faculty, Semnan University, Semnan, Iran.*
E-mail: *mrvpar@semnan.ac.ir*

1. 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. 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 by T. gondii by ingesting infective oocysts that are shed from felids in soil or water. Sporozoites enter the cells and rapidly replicate as tachyzoites. Then, the host immune system is activated, 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 ingesting these infected organs in prey-predator relations. Birds are also a source of protein for humans, and if they are consumed undercooked, human consumers can also be 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 a source of infection for carnivores and humans (Gajadhar, 2015; Chumpolbanchorn et al., 2013). Free-range chickens (Gallus domesticus) have the most potential host for ingesting 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 & 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 protein sources for humans in Semnan City, Iran.

2. Materials and Methods

Sample collection

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

Fifty eggs of free-range chickens and 50 eggs of industrial chickens were also purchased from different stores in Semnan, Iran. Eggs were broken into 50mL sterile tubes, mixed well, labeled, and stored at -20°C 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. Also, 1 mL of homogenized eggs was transferred to 2mL 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 methods. The purified DNA samples were stored in 50mL of TE buffer (10mM Tris and 1mM EDTA, pH 8.) 0 at -20°C.

The extracted DNA of the Toxoplasma RH strain (kindly provided by the 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 primer 1: 5′-GGAACTG-CATCCGTTCATGAG-3′, reverse primer 1 5′-TCTT-TAAAGCGTTCGTGGTC-3′, forward primer 2: 5′-TGCATAGGTTGCAGTCACTG-3′, and reverse primer 2: 5′-GGCGACCAATCTGCGAATACACC-3′ (Burg et al., 1989). Amplification was conducted in 20 µL reaction volumes (ParsTous PCR premix kit, Iran). Then, 10 pmol of each PCR primer (Takapouzist Co. Iran) and 1µL of DNA template (250-500ng) were added to each reaction, and the remaining 20µL 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 at 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 and without 193 bp band). 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 96-bp 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 the Chi-square test in SPSS software. $P<0.05$ was considered a significant difference when comparing the two groups.

3. Results

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

4. Discussion

After observation of relatively high Toxoplasma seroprevalence among free-range chickens (96.7%) and industrial chickens (39.9%) in Semnan, 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.

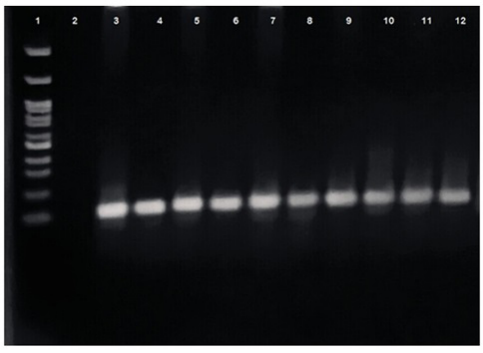

Figure 1. PCR products on agarose gel

lane 1: 100 bp plus molecular marker, lane 2: negative control, lane 3: positive control, lanes 4-12: 96-bp bands of Toxoplasma gondii in some samples.

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

In the USA, 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, the vertical transmission rate of T. gondii to chicken eggs was relatively low (Dubey, 2010). However, after using molecular examinations as sensitive tests, especially nested PCR on the B1 gene (Mason et al., 2010), the infection rate of T. gondii in eggs has been reported to increase. In Iraq, T. gondii DNA has been detected in 20% of free-range chickens (n=30 eggs) (Al-Khanaq et al., 2018), and in Iran, this number was 11% (n=200 eggs) (Khademi et al., 2018). In this study, T. gondii DNA was detected more in eggs than in 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 should be 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, and 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.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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مقاله پژوهشی

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

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

۱. گروه پاتوبیولوژی، دانشکده دامپزشکی، دانشگاه سمنان، سمنان، ایران.
۲. گروه پاتوبیولوژی، دانشکده دامپزشکی شهمیرزاد، دانشگاه سمنان، سمنان، ایران.
۳. گروه علوم بالینی، دانشکده دامپزشکی، دانشگاه سمنان، سمنان، ایران

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چکیده

زمینه مطالعه: توکسوپلاسما گوندی تک یاخته اجباری داخل سلولی در شاخه اپی کمپلکسا می باشد. گربه سانان میزبان نهایی و تمامی حیوانات خونگرم و انسان میزبان‌های واسط توکسوپلاسما محسوب می‌شوند. آلودگی مرغ و تخم مرغ به این انگل عنوان منابع تامین پروتئین انسانی می‌تواند 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 می‌باشد.

روش کار: در این مطالعه ۱۰۰ نمونه ران مرغ گوشتی جمع آوری شده از فروشگاه های سمنان و ۵۰ تخم مرغ بومی و ۵۰ تخم مرغ صنعتی جمع آوری گردید. نمونه ها پس از استخراج DNA مورد ردیابی ژن 1B توکسوپلاسما قرار گرفتند.

نتایج: بر اساس نتایج بدست آمده DNA توکسوپلاسما در ۲۳ درصد (۱۴/۸ - ۳۱/۲ درصد با فاصله اطمینان ۹۵ درصد) نمونه های عضله ران، ۳۶ درصد (۲۲/۷-۴۹/۳ درصد با فاصله اطمینان ۹۵ درصد) تخم مرغ های بومی و ۲۰ درصد (۹-۳۱ درصد با فاصله اطمینان ۹۵ درصد) تخم مرغ های صنعتی ردیابی گردید، میزان آلودگی تخم مرغ های بومی و صنعتی اختلاف معنی داری نداشت (P>۰/۰۵).

نتیجه‌گیری نهایی: بنابراین مشخص گردید که انگل توکسوپلاسما در مرغ‌ها و تخم مرغ های عرضه شده در فروشگاه‌های 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.

کلیدواژه‌ها: رندگان، توکسوپلاسموز، Gallus gallus، شیوع، ماکیان

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* **نویسنده مسئول:**
مریم رسولی
نشانی: گروه پاتوبیولوژی، دانشکده دامپزشکی، دانشگاه سمنان، سمنان، ایران.
رایانامه: *mrvpar@semnan.ac.ir*