Food and Waterborne Parasitology 27 (2022) e00166

Contents lists available at ScienceDirect

Food and Waterborne Parasitology

journal homepage: www.elsevier.com/locate/fawpar

Molecular detection of *Toxoplasma gondii* in chicken hearts from markets and retail stores in Northern Iran

Somayeh Abbaszadeh [a], Aref Teimouri [b], Mohammad Reza Mahmoudi [a], Zahra Atrkar Roushan [c], Nayereh Hajipour [d], Bijan Majidi-Shad [a,*], Meysam Sharifdini [a,*]

[a] Department of Medical Parasitology and Mycology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran
[b] Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[c] Department of Biostatistics, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran
[d] Department of Microbiology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran

ARTICLE INFO

Keywords:
Toxoplasma gondii
Chickens
Northern Iran
B1 gene
SAG1

ABSTRACT

Detection of *Toxoplasma gondii* in chicken products indicates risk of transmission to consumers. The objective of the current study was to investigate the molecular prevalence of *T. gondii* in free-ranging and industrial chickens in Guilan province, Northern Iran. A total of 150 chicken heart samples including 75 free-range and 75 industrial chickens were collected from farmers' markets and chicken retailers in Guilan, Northern Iran, between October 2017 and August 2018. Genomic DNA were extracted from samples and examined for evidence of *T. gondii* using polymerase chain reaction (PCR) targeting the B1 gene. The B1-positive samples were further analyzed by nested-PCR for SAG1 gene. Of the 150 samples, *T. gondii* DNA fragments were detected in 59 (39.3%), including 30 (40%) free-range and 29 (38.7%) industrial chicken. No significant differences of *T. gondii* DNA detection was observed between the free-range and industrial chicken samples ($p = 0.73$). Four selected positive samples were used for amplifying and sequencing of the SAG1 gene. The results revealed that all four sequences of SAG1 had 100% similarity with *T. gondii* sequences previously isolated from an AIDS/HIV patient in Mazandaran province, Northern Iran. Furthermore, the phylogenetic analysis demonstrated that all four sequences were closely related to Type I of *T. gondii*. However, our Type I identification is preliminary and needs to be confirmed by further multilocus sequence typing (MLST) analysis. The findings of the present study provide new data about the presence of *T. gondii* DNA in chicken hearts in the study area. These results confirm that chicken can be used as sentinels for environment contamination; however, further studies are needed to determine the viability of *T. gondii* in chicken hearts from Iran for risk assessment.

1. Introduction

Toxoplasma gondii, the causative agent of toxoplasmosis, is a ubiquitous apicomplexan parasite that ranks among the most common food-borne pathogens (Tenter et al., 2000). Humans become infected mainly by eating raw/undercooked meat containing *T. gondii*

* Corresponding authors.
E-mail addresses: bmajidis39@gmail.com (B. Majidi-Shad), sharifdini@gums.ac.ir (M. Sharifdini).

https://doi.org/10.1016/j.fawpar.2022.e00166
Received 27 December 2021; Received in revised form 22 May 2022; Accepted 31 May 2022
Available online 3 June 2022

tissue cysts or through accidental consumption of food and/or water contaminated with environmental oocysts excreted in cat feces. Vertical transmission of tachyzoites from the pregnant mother to developing fetus is another route of human infection (Montoya and Liesenfeld, 2004). The primary infection in immunocompetent individuals usually result in mild and mononucleosis-like symptoms while it could result in life-threatening and severe conditions in immunocompromised persons such as patients with human immunodeficiency virus (HIV) infection/acquired immune deficiency syndrome (AIDS), cancer patients and those undergoing organ transplantation (Dubey, 2010a). Furthermore, primary infection during pregnancy might pose severe threats to the fetus, such as retinochoroiditis and serious developmental disorders such as hydrocephaly, microcephaly, and intellectual disability (Teimouri et al., 2020). Moreover, spontaneous abortion, prematurity, and stillbirth can arise (Shojaee et al., 2018; Shahighi et al., 2021).

T. gondii infection among poultry, especially in free-range, is considered a good indicator of environmental contamination with sporulated oocysts of *T. gondii* (More et al., 2012; Dubey et al., 2015). High rates of infection have been reported in chickens raised in backyards (up to 100%) and free-range organic (30–50%) worldwide. However, toxoplasmosis can cause clinical disease in chickens rarely (Dubey, 2010b). The first report of toxoplasmosis in chickens was a case of an infected hen in Germany in 1939 (Hepding, 1939) that was followed by numerous cases in other countries (Dubey, 2010b; Wang et al., 2020). An early study on toxoplasmosis in domestic fowls from Iran involved 162 serum samples; *T. gondii* isolated from six of 109 (5.4%) chicken and the reported overall prevalence was 29% based on an indirect hemagglutination antibody (IHA) test (Ghorbani and Gharavi, 1990). In a later large-scale investigation, a prevalence of 10.04% was found among 697 samples from free-ranging, semi-industrial and industrial chickens in southern Iran (Asgari et al., 2008).

Chicken meat are usually sufficiently heated before consumption, assuring the inactivation of *T. gondii* tissue cysts (Cook et al., 2000). However, unique dishes or products such as chicken carpaccio, chicken sashimi, barbecued chicken, sausages, livers, and hearts might be consumed raw or inadequately heated to kill the parasite before consumption. Furthermore, tasting meat or products during cooking and poor kitchen hygiene have been reported as risk factors for human infection (Kapperud, 1994; Cook et al., 2000). As well, improper handling of slaughter residues could facilitate the transmission of the *T. gondii* from infected chickens to domestic cats and hence further propagation of the infection to other vertebrate intermediate hosts via oocysts shed by cats (Schares et al., 2017). In Iran, chicken hearts are sold in markets and by chicken retailers. The aim of present study was to assess the molecular prevalence of *T. gondii* in free-ranging and industrial chicken hearts collected from farmers' markets and chicken retailers in Guilan province, Northern Iran.

2. Materials and methods

2.1. Ethics approval and consent to participate

The study was approved by Ethics Committee of Guilan University of Medical Sciences, Iran (IR.GUMS.REC.1398.513).

2.2. Sample collection

This survey was a cross-sectional study conducted from October 2017 to August 2018. A total of 150 chicken heart samples were collected in Guilan, Iran, including 75 free-range and 75 industrial chickens. Free range chickens were raised in backyards of villages and purchased at farmers' markets before their hearts were removed and collected as specimen. The industrial chickens, were commercially raised in large numbers in confined systems. Their hearts were collected from retail stores. All heart samples were freshly collected and were placed individually in zipped plastic bags, and kept cool during transportation to the laboratory where they were stored without preservatives at −20 °C until DNA was extracted.

2.3. DNA extraction

Each heart sample was chopped and homogenized, and 25–50 mg removed for genomic DNA extraction by a commercial extraction kit (Viragen, Iran) according to manufacturer's instructions. A sample of DNA extracted from a negative chicken heart was used as a control for contamination in each round of DNA extraction. All extracted DNA were stored at −20 °C for polymerase chain reaction (PCR) amplification. The extracted DNA was quantified using a NanoDrop spectrophotometer (Thermo Scientific, Walther, MA, USA), and the purity was checked by estimating the A260/A280 ratio. The reference DNA was extracted from *T. gondii* RH strain tachyzoites (Teimouri et al., 2019).

2.4. PCR targeting B1 gene

PCR reactions were performed in a 30 μL final volume containing 2 × red PCR premix (Ampliqon, Odense, Denmark), 20 pmol of each primer, and 3 μL of extracted DNA. A 469-base pair (bp) fragment of B1 gene was amplified using Tg1 (forward: 5′-AAAAATGTGGGAATGAAAGAG-3′) and Tg2 (reverse: 5′-ACGAATCAACGGAACTGTAAT-3′) primers (Jalal et al., 2004). The PCR reactions were amplified using a thermal cycler (Eppendorf 5331, Germany) with the following cycling conditions: initial denaturation step at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 50 °C for 45 s and extension at 72 °C for 1 min. The final extension was carried out at 72 °C for 7 min. The PCR product was analyzed by electrophoresis on 1.5% agarose gel containing Safe DNA Gel Stain (SinaClon, Iran) and visualized under UV illumination. The reference *T. gondii* DNA and double distilled water were used as positive and no template controls, respectively in each run.

2.5. Nested-PCR targeted SAG1 gene

The B1-positive samples were further analyzed by nested-PCR for SAG1 gene. The SAG1 is one of the most immunogenic and stage-specific *T. gondii* antigens, which were used extensively as a genotyping and diagnostic marker of *T. gondii* (Ivović et al., 2012; Teimouri et al., 2021). Nested-PCR targeting a 390 bp region of the SAG1 gene was performed using primary and secondary primers as previously described by (Ribeiro et al., 2015).

2.6. Sequencing and phylogenetic analysis

A number of SAG1 gene amplified products were selected and sequenced on an ABI 3730 automatic sequencer (Applied Biosystems, Foster City, CA, USA) using the same primers as used in the PCR reaction. Before sequencing of PCR products, amplified DNA from PCRs was purified using Accuprep Gel purification Kit (Bioneer, Deajeon, Korea) according to the manufacturer's instructions. The resulting sequences were edited using Chromas v.2.01 and the basic local alignment search tool (BLAST) program (http://www.ncbi.nlm.nih.gov/blast/) was used to compare the consensus sequences with GenBank references sequences. A phylogenetic tree was constructed with sequences obtained in the present study along with reference sequences deposited in GenBank using the maximum-likelihood (ML) method and Tamura-3-parameter model and genetic distances were calculated with Maximum Composite Likelihood model in MEGA6 software (http://www.megasoftware.net/) (Saitou and Nei, 1987). The reliability of the phylogenetic trees were assessed using the bootstrap value with 1000 replications.

2.7. Statistical analysis

Statistical analysis was carried out with SPSS Softwarev.16.0 (IBM Analytics, USA). Chi-square test was used to assess whether there were significant differences between occurrence of the *T. gondii* DNA in hearts from free-range and industrial chickens. *P*-values less than 0.05 were reported statistically significant.

3. Results

3.1. Molecular detection of T. gondii

Of the 150 free-range and industrial chicken heart samples, *T. gondii* DNA fragments from the B1 gene were detected in 59 (39.3%) samples, including 30 (40%) free-range and 29 (38.7%) industrial heart chicken samples (Table 1). Chi-square analysis showed no significant differences of *T. gondii* infection between the free-range and industrial chicken samples ($p = 0.73$). The odds ratio (OR) of using each type of chicken is almost the same for consumers (OR = 1.1, CI95 = 0.86–1.66).

3.2. Sequences and phylogenetic tree analysis

Four amplified SAG1 gene products from two isolates of free-range chicken and two isolates of industrial chicken were sequenced. All sequences were registered in the GenBank database with accession numbers: MW553321 to MW553324. All sequences were compared to the GenBank database and the results revealed all four sequences of SAG1 had 100% similarity with *T. gondii* isolated from an AIDS/HIV patient (accession number: MH704617) in Mazandaran province, Northern Iran. The phylogenetic analysis demonstrated that our four sequences were similar and closely related to Type I strain of *T. gondii*. The phylogenetic tree also illustrated that our sequences were clustered with *T. gondii* isolates obtained from humans and sheep in Mazandaran province, Northern Iran (See Fig. 1).

4. Discussion

In the current study, *Toxoplasma* DNA was detected in chicken heart samples sold for human consumption from various markets located in the Guilan province, Northern Iran. DNA of *T. gondii* was detected in 59 out of 150 tested samples (39.33%) using a PCR assay targeting the B1 gene. Several studies have shown that the heart is the preferred organ for isolating viable *T. gondii* in chickens (Dubey et al., 2020). Various types of edible birds such as turkey, starling, duck, goose, and ringdove have been examined for *T. gondii* worldwide (Dubey, 2010b). However, according to a recent review, few such studies have been carried out in Iran and information on type identification is unavailable (Shokri et al., 2017). In a survey on 39 starlings, five (12.8%) were infected with *T. gondii* (Khademvatan et al., 2013) and another study in Kerman province revealed that 4.8% of 332 ringdoves were infected (Keshavarz Valian

Table 1
Toxoplasma gondii infection detected in free-range and industrial chicken heart samples using PCR assay based on B1 gene.

Sample	Positive (%)	Negative (%)	Total (%)	P value
Free-range chicken	30 (40)	45 (60)	75 (100)	0.73
Industrial chicken	29 (38.7)	46 (61.3)	75 (100)	0.73
Total	59 (39.3)	91 (60.7)	150 (100)	–

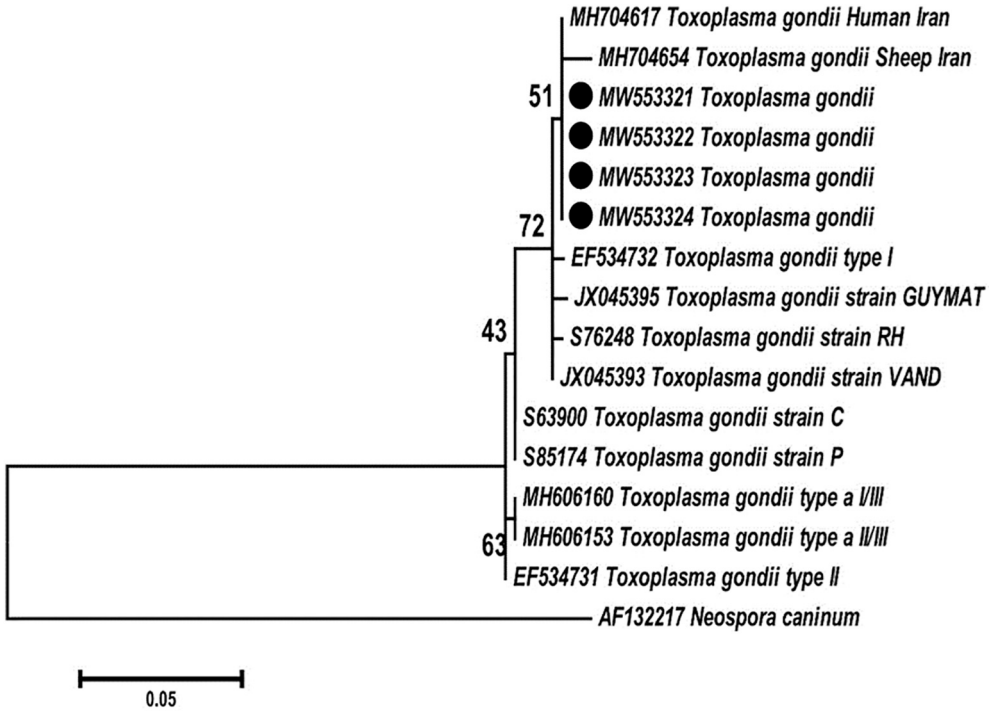

Fig. 1. Phylogenetic tree obtained via the Maximum Likelihood (ML) method and the Hasegawa-Kishino-Yano model based on the SAG1 gene (The black circle indicates the sequence derived from this study). The numbers above branches correspond to bootstrap values based on 1000 replicates. *Neospora caninum* served as an outgroup. Scale bar represented 0.05 changes per nucleotide.

and Ebrahimi, 1993). A study on 54 turkeys showed that 47 (87%) were infected with *T. gondii* (Sarkari et al., 2014). It seems that turkeys are more likely to be infected with *T. gondii* than chickens or other avian species; however additional studies should be conducted to confirm this difference. The lowest prevalence of toxoplasmosis was observed in a study on 125 rooks (Eslami et al., 2007) in which only 1.6% was found to be infected. Rooks rarely feed on grains from the ground and could be a possible factor in the low prevalence of *T. gondii* infection in birds (Shokri et al., 2017).

In the current study, 30 (40%) free-range and 29 (38.7%) industrial chicken samples were infected with *T. gondii*. Statistical analysis showed no significant differences of *T. gondii* DNA detection between the free-range and industrial chickens ($p = 0.73$). Both may be due to the possibility of contamination of poultry feed with *T. gondii* oocysts or the presence of cats in their holding. Conversely, in several studies, the differences were significant as fewer industrial chickens were infected than those in free-range due to the differences in contamination of food and water sources with *T. gondii* oocysts (Shokri et al., 2017). A study by Asgari et al., 2008 using IFA serology reported that the *T. gondii* infection rates in free-ranging chicken, semi-industrial, and industrial poultry farms were statistically significantly different at 27.1%, 12%, and 2.02%, respectively. An international study demonstrated that the prevalence of infection in chickens varies in different countries ranging from 0.01% in the Czech Republic to 71.3% in Italy, with a higher rate of infection in free-ranging chickens compared to other types of chickens (Dubey, 2010b). The lower infection rates in industrial chickens may be due to their feeding habit that likely reduces contact with cats and other Felidae. Environmental control in free-range chicken is challenging due to unrestricted stray cats and potential intermediate hosts (Dubey, 2010b).

The phylogenetic analysis demonstrated that all four sequences were closely related to Type I of *T. gondii*. However, the Type I identification in this study is considered preliminary as genotyping with a single marker does not allow for identification of nonclonal strains. More precisely, determination of the presence of polymorphisms in the population, application of multilocus PCR-RFLP, and multilocus sequence typing (MLST) analysis are necessary (Su et al., 2006; Ivović et al., 2012). All major genotypes (I, II, III) of *T. gondii* were reported from chickens in different area of the world (Dubey et al., 2002; Dubey et al., 2003a; Dubey et al., 2003b; Sreekumar et al., 2003). Supported by our data, Type I was the predominant genotype of *T. gondii* from chickens in northwestern Iran (Mahami-Oskouei et al., 2017). Additionally, a similar study from Brazil reported that Type I was a common *T. gondii* genotype in free-range chickens (Dubey et al., 2002). In contrast, several studies in Iran reported genotypes II and III *T. gondii* with no evidence of Type I from birds (Zia-Ali et al., 2005; Zia-Ali et al., 2007; Khademvatan et al., 2013). In a study conducted in Mazandaran province, Northern Iran, genotyping of *T. gondii* isolates revealed only Type III using Multiplex PCR for 5 microsatellite markers (Zia-Ali et al., 2005). The results of another study carried out in southwest of Iran, revealed that genotyping of *T. gondii* isolates obtained from infected birds were Type II (19.5%) and III (80.5%) (Khademvatan et al., 2013). The diversity of *T. gondii* genotypes infecting chickens in Iran may be due to different geographical and ecological conditions in various regions of the country. This study also showed that all four sequences of SAG1 had 100% match with *T. gondii* sequences isolated from an AIDS/HIV patient (accession number: MH704617) and 99.7% with

isolates from an aborted sheep fetus (accession number: MH704654) in Northern Iran. The main limitation of this study is that the molecular identification is based on the sequencing results of only one marker (SAG1). Another limitation of our study is that we found the parasite DNA in the hearts of chickens but did not isolate live *T. gondii* from the samples; further studies are needed to determine the viability of *T. gondii* in chicken hearts from Iran.

5. Conclusions

These findings provide new data on the presence of *T. gondii* DNA in chicken hearts in Guilan province in Iran and can be used in quantitative microbial risk assessments of foodborne toxoplasmosis in Iran. These results indicate that chickens can be used as sentinels for environment contamination; however, further studies are needed to determine the viability of *T. gondii* in chicken hearts from Iran for risk assessment.

Declaration of Competing Interest

The authors declare that we do not have any conflict of interests.

Acknowledgments

We would like to express our thanks to the facilities and financial support (grant No: 98092306) provided by the School of Medicine, Guilan University of Medical Sciences, Rasht, Iran.

References

Asgari, Q., Akrami Mohajeri, F., Kalantari, M., Esmaeilzadeh, B., Farzaneh, A., Moazeni, M., 2008. Chicken toxoplasmosis in different types of breeding: a seroprevalence survey in southern Iran. Int. J. Poult. Sci. 7 (12), 1247–1250.

Cook, A.J., Gilbert, R.E., Buffolano, W., Zufferey, J., Petersen, E., Jenum, P.A., Foulon, W., Semprini, A.E., Dunn, D.T., 2000. Sources of *Toxoplasma* infection in pregnant women: European multicentre case-control study. European research network on congenital toxoplasmosis. BMJ. 321, 142–147.

Dubey, J.P., 2010a. Toxoplasmosis of Animals and Humans, 2nd ed. CRC Press LLC, Boca Raton, FL.

Dubey, J.P., 2010b. *Toxoplasma gondii* infections in chickens (*Gallus domesticus*): prevalence, clinical disease, diagnosis and public health significance. Zoonoses Public Health 57, 60–73.

Dubey, J.P., Graham, D.H., Blackston, C.R., Lehmann, T., Gennari, S.M., et al., 2002. Biological and genetic characterisation of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*) from Sa~o Paulo, Brazil: unexpected findings. Int. J. Parasitol. 32, 99–105.

Dubey, J.P., Graham, D.H., Dahl, E., Hilali, M., El-Ghaysh, A., Sreekumar, C., Kwok, O.C.H., Shen, S.K., Lehmann, T., 2003a. Isolation and molecular characterization of *Toxoplasma gondii* from chickens and ducks from Egypt. Vet. Parasitol. 114, 89–95.

Dubey, J.P., Graham, D.H., Dahl, E., Sreekumar, C., Lehmann, T., Davis, M.F., Morishita, T.Y., 2003b. *Toxoplasma gondii* isolates from free-ranging chickens from the United States. J. Parasitol. 89, 1060–1062.

Dubey, J.P., Lehmann, T., Lautner, F., Kwok, O.C., Gamble, H.R., 2015. 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. 214, 55–58.

Dubey, J.P., Pena, H., Cerqueira-Cézar, C.K., Murata, F., Kwok, O., Yang, Y.R., Gennari, S.M., Su, C., 2020. Epidemiologic significance of *Toxoplasma gondii* infections in chickens (Gallus domesticus): the past decade. Parasitology 147 (12), 1263–1289. https://doi.org/10.1017/S0031182020001134.

Eslami, A., Meshgi, B., Rahbari, S., Ghaemi, P., Aghaebrahimi- Samani, R., 2007. Biodiversity and prevalence of parasites of rook (*Corvus frugilegus*) in Iran. Iran. J. Parasitol. 2 (4), 42–43.

Ghorbani, M., Gharavi, M.J., 1990. Serological and parasitological investigations on toxoplasma infection in domesteic fowls in Iran. Iran. J. Public Health 19, 9–17.

Hepding, L., 1939. Über Toxoplasmen (*Toxoplasma gallinarum* n. sp.) in der retina eines Huhne and über deren Beziehung zur Hühnerlähmung. Zeitschr. Infektkr. 55, 109–116.

Ivović, V., Vujanić, M., Živković, T., Klun, I., Djurković-Djaković, O., 2012. Molecular detection and genotyping of toxoplasma gondii from clinical samples. In: Djurković-Djaković, O. (Ed.), Toxoplasmosis—Recent Advances. InTech, Rijeka, Croatia, pp. 103–119.

Jalal, S., Nord, C.E., Lappalainen, M., Evengard, B., 2004. Rapid and sensitive diagnosis of *toxoplasma gondii* infections by PCR. Clin. Microbiol. Infect. 10, 937–939.

Kapperud, G., 1994. Campylobacter infections. Epidemiology, risk factors and preventive measures (in Norwegian). Tidskr Nor Lægeforen. 114, 795–799.

Keshavarz Valian, H., Ebrahimi, A., 1993. Toxoplasmosis among birds in Kerman. In: Proceeding of the 2th Iranian National Congress of Zoonosis. May 25–27. Tabriz University of Medical Sciences, Iran.

Khademvatan, S., Saki, J., Yousefi, E., Abdizadeh, R., 2013. Detection and genotyping of *toxoplasma gondii* strains isolated from birds in the southwest of Iran. Br. Poult. Sci. 54 (1), 76–80.

Mahami-Oskouei, M., Moradi, M., Fallah, E., Hamidi, F., Akbari, Asl Rahnamaye, N., 2017. Molecular detection and genotyping of *toxoplasma gondii* in chicken, beef, and lamb meat consumed in northwestern Iran. Iran. J. Parasitol. 12 (1), 38–45.

Montoya, J.G., Liesenfeld, O., 2004. Toxoplasmosis. Lancet 363, 1965–1976. https://doi.org/10.1016/S0140-6736(04)16412-X.

More, G., Maksimov, P., Pardini, L., Herrmann, D.C., Bacigalupe, D., Maksimov, A., Basso, W., Conraths, F.J., Schares, G., Venturini, M.C., 2012. *Toxoplasma gondii* infection in sentinel and free-range chickens from Argentina. Vet. Parasitol. 184, 116–121.

Ribeiro, L., Santos, L., Brito, Jr.P., Maciel, B., Da Silva, A., Albuquerque, G., 2015. Detection of *toxoplasma gondii* DNA in Brazilian oysters (*Crassostrea rhizophorae*). Genet. Mol. Res. 14 (2), 4658–4665.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4 (4), 406–425.

Sarkari, B., Asgari, Q., Bagherian, N., Ashkani Esfahani, S., Kalantari, M., Mohammadpour, I., et al., 2014. Molecular and serological evaluation of *Toxoplasma gondii* infection in reared turkeys in Fars Province. Iran Jundishapur J. Microbiol. 7 (7), 11598.

Schares, G., Bangoura, B., Randau, F., Goroll, T., Ludewig, M., Maksimov, P., Matzkeit, B., Sens, M., Barwald, A., Conraths, F.J., Opsteegh, M., Van der Giessen, J., 2017. High seroprevalence of *Toxoplasma gondii* and probability of detecting tissue cysts in backyard laying hens compared with hens from large free-range farms. Int. J. Parasitol. 47, 765–777.

Shahighi, M., Heidari, A., Keshavarz, H., Bairami, A., Shojaee, S., et al., 2021. Seroepidemiological study of toxoplasmosis in women referred to a pre-marriage counseling center in Alborz Province, Iran. BMC Res Notes. 30, 14(1):163. https://doi.org/10.1186/s13104-021-05581-0.

Shojaee, S., Teimouri, A., Keshavarz, H., Azami, S.J., Nouri, S., 2018. The relation of secondary sex ratio and miscarriage history with *Toxoplasma gondii* infection. BMC Infect. Dis. 18, 307. https://doi.org/10.1186/s12879-018 -3228-0.

Shokri, A., Sharif, M., Teshnizi, S.H., Sarvi, S., Rahimi, M.T., et al., 2017. Birds and poultries toxoplasmosis in Iran: a systematic review and meta-analysis. Asian Pac J Trop Med 10, 635–642.

Sreekumar, C., Graham, D.H., Dahl, E., Lehmann, T., Raman, M., Bhalerao, D.P., Vianna, M.C., Dubey, J.P., 2003. Genotyping of *Toxoplasma gondii* isolates from chickens from India. Vet. Parasitol. 118, 187–194.

Su, C., Zhang, X., Dubey, J.P., 2006. Genotyping of *Toxoplasma gondii* by multilocus PCR-RFLP markers: a high resolution and simple method for identification of parasites. Int. J. Parasitol. 36, 841–848.

Teimouri, A., Modarressi, M.H., Shojaee, S., Mohebali, M., Rezaian, M., Keshavarz, H., 2019. Development, optimization, and validation of an in-house dot-ELISA rapid test based on SAG1 and GRA7 proteins for serological detection of *Toxoplasma gondii* infections. Infect. Drug Resist. 12, 2657–2669. https://doi.org/10.2147/IDR.S219281.

Teimouri, A., Mohtasebi, S., Kazemirad, E., Keshavarz, H., 2020. Role of *Toxoplasma gondii* IgG avidity testing in discriminating between acute and chronic toxoplasmosis in pregnancy. J. Clin. Microbiol. 58, e00505–e00520. https://doi.org/10.1128/JCM.00505-20.

Teimouri, A., Abbaszadeh Afshar, M.J., Mohtasebi, S., Jafarpour Azami, S., Alimi, R., Keshavarz, H., 2021. Assessment of an in-house enzyme-linked immunosorbent assay and IgG avidity test based on SAG1 and GRA7 proteins for discriminating between acute and chronic toxoplasmosis in humans. J. Clin. Microbiol. 59 (8), e0041621 https://doi.org/10.1128/JCM.00416-21.

Tenter, A.M., Heckeroth, A.R., Weiss, L.M., 2000. *Toxoplasma gondii*: from animals to humans. Int. J. Parasitol. 30, 1217–1258. https://doi.org/10.1016/S0020-7519(00)00124-7.

Wang, R., Wang, N., Zhang, H., Wang, F., Li, H., Liu, Y., et al., 2020. Prevalence of *toxoplasma gondii* infections in chicken hearts from farmers' markets and supermarkets in the Tai'an Region of China. J. Food Prot. 22 (2), 338–341. https://doi.org/10.4315/0362-028X.JFP-19–374, 38.

Zia-Ali, N., Keshavarz-Valian, H., Rezaian, M., Khorramizadeh, M.R., Kazemi, B., Fazaeli, A., Darde, M., 2005. Molecular characterization of *Toxoplasma gondii* from bird hosts. Iran. J. Public Health 34, 27–30.

Zia-Ali, N., Fazaeli, A., Khoramizadeh, M., Ajzenberg, D., Dard'e, M., et al., 2007. Isolation and molecular characterization of *Toxoplasma gondii* strains from different hosts in Iran. Parasitol. Res. 101 (1), 111–115.